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AUS DEM SERO-BAKTERIOLOGISCHEN INSTITUT DER UNIVERSITÄT TURKU

VERGLEICHE ZWISCHEN DEM LÖFFLERSCHEN UND DEM VON HERRMANN MODIFIZIERTEN CLAUBERG III-NÄHRBODEN IN DER BAKTERIOLOGISCHEN DIAGNOSTIK DER DIPHTHERIE

Von

KEIJO JÄNTTI

(Eingegangen bei der Redaktion am 19. April 1949)

Der in Löfflers Diphtheriebazillendiagnostik angewandte Serumnährboden hat allmählich neuen kaliumtellurithaltigen Nährböden weichen müssen. Die von diesen erhaltenen Ergebnisse sind besser gewesen als die des Löfflerschen Verfahrens, das jedoch weiter noch als schnelles Hilfs- und Kontrollmittel angewandt wird.

Von den kaliumtellurithaltigen dürften in der Praxis die allgemeinsten Claubergs sog. II und III und Mc Leods Nährböden sowie die von HERRMANN entwickelte Modifikation des Clauberg III-Nährbodens sein. RANTASALO (5) hat in den letzten Jahren ein Verfahren entwickelt, bei dem neben dem Kaliumtellurit Natriumfluorid angewandt wird.

Bei dem Clauberg III-Nährboden (1) ebenso wie bei der von HERRMANN an ihm gemachten Modifikation ist der wesentlichste Teil das sog. Indikatorsystem, Glukose und Wasserblau-Farbstoff, welches das Ablesen der Ergebnisse mit blossem Auge erleichtert, indem die Diphtheriebazillenkolonie sich unter seiner Einwirkung hellblau färbt. Der Clauberg III-Nährboden war jedoch nicht so gut, wie man gemeint hatte, denn es wurde bemerkt, dass man mit ihm in 8—10% der Fälle negative Ergebnisse bekam in Fällen, wo man mit dem Löfflerschen Verfahren positive erhielt (3).

Bei der Ermittlung der Ursachen hierfür entwickelte HERRMANN seine Modifikation, die sich vom Clauberg III-Nährboden im wesentlichsten dadurch unterscheidet, dass die Kaliumtelluritmenge um 100%, die Serummenge um 10% vermehrt und das dem Wachstum des Diphtheriebazillus schädliche Metachromgelb weggelassen ist.

In seiner Untersuchungsreihe, die über 100,000 Proben enthält, erhielt HERRMANN, indem er sein eigenes und das Löfflersche Verfahren nebeneinander anwandte und die positiven Ergebnisse durch Blutagarplattenkulturen und Typenbestimmungen kontrollierte, 2,340 St. positive Ergebnisse. Die mit den verschiedenen Verfahren erzielten Ergebnisse verteilten sich wie folgt:

Löffler +, Herrmann-Clauberg + ...	1,948 St.	83.2%
Löffler —, Herrmann-Clauberg + ...	366 »	15.7%
Löffler +, Herrmann-Clauberg —	26 »	1.1%

Bei blosser Anwendung des Löfflerschen Verfahrens wären somit 15.7% weniger positive Ergebnisse erzielt worden.

OUCHTERLONY (4) hat bei Anwendung derselben Verfahren wie HERRMANN von 2,694 Proben 184 positive gewonnen wie folgt:

Löffler + (oder +?), Herrmann-Clauberg +	53 St.	29%
Löffler —, Herrmann-Clauberg + ...	127 »	69%
Löffler +, Herrmann-Clauberg —	4 »	2%

Bei blosser Anwendung des Löfflerschen Verfahrens wären 69% weniger positive Ergebnisse erzielt worden.

In unserem Institut in Turku ist der von HERRMANN modifizierte Clauberg III-Nährboden seit Frühjahr 1945 neben dem Löfflerschen Verfahren angewandt worden. Die Schwierigkeit, bestimmte Chemikalien zu bekommen, hat die Anwendung des ersteren einigermassen beschränkt. Die Ergebnisse beider Kulturen sind nach Verlauf von 20 und 44 Stunden abgelesen worden. Von den Herrmann-Clauberg-Nährböden, die ein positives Resultat ergeben haben, ist eine nachfolgende Untersuchung auf Agarplatten mit 5% Schafsblut gemacht worden, davon auch ein Mikroskoppräparat und eine Diphtheriebazillentypenbestimmung mit Kohlenhydratreihen. Bei den Proben, bei denen mit dem Herrmann-Clauberg-Nährboden ein negatives, aber mit dem Löfflerschen Verfahren ein positives Ergebnis erzielt wurde, ist von den positiven Ergeb-

nissen des Löfflerschen Verfahrens, wenn es möglich gewesen ist, eine weitere Kultur auf einer neuen Herrmann-Clauberg Platte gemacht worden; sofern jetzt ein positives Ergebnis erzielt wurde, ist von den letzteren eine weitere Untersuchung auf einer Schafsblutplatte und davon eine Typenbestimmung gemacht worden. — Auf diese Weise sind von 13,713 untersuchten Proben, von denen einen bedeutenden Teil die im Turkuer Epidemiekrankenhaus von alten Fällen genommenen bildeten, 1,885 positive Ergebnisse gewonnen worden. Die endgültige Antwort ist also auf Grund der Blutplattenuntersuchung und der Typenbestimmung gegeben worden. Auf die verschiedenen Verfahren haben sich die Ergebnisse wie folgt verteilt:

Löffler +, Herrmann-Clauberg + ...	1,116 St.	59.2%
Löffler —, Herrmann-Clauberg + ...	677 »	35.9%
Löffler +, Herrmann-Clauberg —	92 »	4.9%

Bei blosser Anwendung des Löfflerschen Verfahrens hätten wir in 35.9% der Fälle falsche negative Ergebnisse erhalten, mit anderen Worten mehr als HERRMANN (15.7%), aber weniger als OUCHTERLONY (69%). Bei blosser Anwendung des Herrmann-Clauberg Verfahrens hätten wir in 4.9% der Fälle falsche negative Ergebnisse bekommen, also mehr als HERRMANN (1.1%) und OUCHTERLONY (2%).

Ausser diesen unseren 1,885 positiven Ergebnissen hat sich eine Probe, die sich mit dem Herrmann-Clauberg Verfahren als positiv, mit dem Löfflerschen als negativ erwiesen hat, durch Blutplattenuntersuchung in 119 Fällen als negativ herausgestellt. Positive Ergebnisse nur mit dem Herrmann-Clauberg Nährboden sind also erzielt worden

$$1{,}116 + 677 + 119 = 1{,}912 \text{ St.},$$

von ihnen sind falsche positive gewesen 119 St. bzw. 6.2%.

Mit dem Herrmann-Clauberg Verfahren haben wir bedeutend mehr positive Ergebnisse erhalten als mit dem Löfflerschen Verfahren, aber andererseits hat das Herrmann-Clauberg Verfahren ziemlich viele (etwa 11%) falsche positive oder negative Ergebnisse gebracht. Es besteht also Grund, das Löfflersche Verfahren neben dem Herrmann-Clauberg Verfahren anzuwenden ebenso wie die Blutagarplattenuntersuchung zur Kontrolle des letzteren.

ZUSAMMENFASSUNG

13,713 Diphtherieproben sind untersucht worden, wobei als Nährböden der Löfflersche und der von HERRMANN modifizierte Clauberg III-Nährboden gleichzeitig angewandt und insgesamt 1,885 positive Ergebnisse erzielt worden sind. Von diesen hat das Löfflersche Verfahren in 35.9%, das Herrmann-Claubergsche in 4.9% der Fälle ein negatives Ergebnis gebracht. Mit dem letzteren sind in 6 % der Fälle falsche positive Ergebnisse erzielt worden. Es scheint somit Grund zu bestehen, neben dem Herrmann-Clauberg Nährboden als ergänzende Mittel das Löfflersche Verfahren und Blutagarreinkulturen anzuwenden.

LITERATUR

1. CLAUBERG, K. W.: Zbl. Bakter. usw. I Orig. 1935:*134*:271.
2 HERRMANN, W.: Z. Hyg. usw. 1939:*121*:540.
3. HERRMANN, W.: Zbl. Bakter. usw. I Orig. 1941:*147*:298.
4. OUCHTERLONY, Ö.: Nordisk Hygienisk Tidskrift 1946:*27*:182.
5. RANTASALO, I.: Ann. Med. Exp. et Biol. Fenn. 1948:Suppl. 7.

FROM THE WIHURI RESEARCH INSTITUTE

EFFECT OF DILATATION AND INFLAMMATION OF THE GALLBLADDER UPON THE ELECTROCARDIOGRAM OF TEST ANIMALS

By

TEPPO VARTIO

(Received for publication April 20, 1949)

A fact which has long been known is that inflammation of the gallbladder, as well as gallstones, occur more frequently in persons with myocardial lesions and coronary sclerosis than in healthy individuals. Conversely, persons with a diseased gallbladder show a higher incidence of myocardial lesions and coronary sclerosis than healthy individuals. BROCKBANK (1898) encountered, as post-mortem findings, gallstones in 10.9 per cent of persons suffering from chronic cardiac disease, but only in 5.4 per cent of those with normal hearts. WILLIUS and BROWN (1924) found chronic cholecystitis in 26 per cent of 86 autopsies of persons with coronary sclerosis. In accord with this are the results obtained by TENNANT and ZIMMERMAN (1931), WILHELMY and HELWIG (1935), BREYFOGLE (1940), etc. SCHWARTZ and HERMAN (1931) were able to demonstrate myocardial lesions in 63 per cent of 109 patients with cholecystitis, but in only 41 per cent of an equal number of patients free from gallbladder disturbances. Myocardial lesions were found by LAIRD (1938) in 77 per cent of 65 patients suffering from gallbladder disease. MILLER (1932) noted a higher incidence of coronary sclerosis in patients with cholecystitis (with or without calculi) than in healthy individuals.

The following factors have been advanced to explain the mechanism of this coincidence: common disturbance of the lipid-

cholesterine metabolism affecting both the heart and gallbladder (Bean 1937), the noxious influence of bile substance upon the myocardium (Babcock 1909, Vest 1934), the inflamed gallbladder, constituting a focus of infection, which causes damage to the heart with its toxins (Babcock 1909, Rolleston 1920, Boyd 1928 and Campbell 1936), or reflexogenic influences transmitted from the gallbladder to the heart (Buchbinder 1930, Bettman and Rubinfeld 1935, Constancinescu and Tuchel 1939, et al.)

As concerns the reflex connection between the gallbladder and the heart, it is held that in response to irritation from the gallbladder contraction of the coronary vessels will take place (Danielopolu 1939), the pathway for the afferent impulse being the phrenicus, the vagus or the sympathicus (Vest 1934). A number of experiments have been carried out to clarify this process. Buchbinder (1930) noted in frogs a well-nigh specific reflex, i.e., that speedy incision of the gallbladder and subsequent drainage have been accompanied by a complete standstill for a few seconds in the function of the heart. As this reflex can be inhibited by the administration of atropine, by vagotomy or decapitation, there is evidence of the vagus being the pathway along which the impulse travels. Hodge, Messer, and Hill (1947) carried out dilatation of the gallbladder in dogs with normal hearts, as well as in dogs with experimentally induced cardiac infarcts. The former showed no changes in the electrocardiogram, whereas the latter presented various alterations of the QRS complex, which, according to the writers were suggestive of changes in the coronary circulation. Prior to them, Bellet (1938) obtained much the same results. Taquini, Yodice, and Taquini (1947) made similar experiments without finding any appreciable changes in the electrocardiogram. By measuring in dogs the coronary circulation volume by aid of a Morawitz cannula, Hinrichsen and Ivy (1933) observed in the majority of cases an increase in the coronary circulation upon dilatation of the gallbladder. Donoso and Steiner (1946) also found dilatation of the gallbladder of the dog to be accompanied by an increased coronary circulation as measured with a Morawitz cannula, whereas emptying the gallbladder by manual pressure resulted in a reduction of the coronary circulation in 77 per cent of the cases. Eckenhoff, Hafkenschiel, and Landmesser (1947) carried out dilatation of the gallbladder in dogs with an inserted rubber ball and subsequently determined the coronary

circulation volume by aid of a bubble flowmeter, but failed to see alterations in the coronary circulation upon distention of the gallbladder. BETTMAN and RUBINFELD (1935) took electrocardiograms in man during operation on the gallbladder and noted an increase in the frequency as well as the presence of extrasystoles on stretching and pressing the gallbladder. MC ARTHUR and WAKEFIELD (1944) distended the gallbladder in man during operation, their finding being various alterations in the electrocardiogram, such as a rise in the frequency, lowering of the T wave to the isoelectric level, extrasystoles, and a prolonged P-R interval, but no signs indicating coronary insufficiency.

The effect exerted upon the heart by an infected gallbladder is very little dealt with in the literature. A constant change, but not a permanent one, was observed by CLARKE (1945) in the T wave of patients with active gallbladder inflammation. According to him inflammation of the gallbladder produces irritation and spasticity in the adjacent tissues and this again produces irritation reflexes, which, referred into the coronary vascular bed by the pathways of the autonomic nervous system, finally result in contraction of its vessels. My search in the literature revealed no mention of earlier experimental study of this matter.

OWN INVESTIGATIONS

The present study was directed to possible reactions of the heart, reflex in type, attributable to dilatation and contraction of the gallbladder and to the effect upon the heart of experimentally induced cholecystitis, as expressed in the electrocardiogram.

The research methods were as follows: — Rabbits and cats served as test animals. The electrocardiograms were taken with the test animals supine and fastened to the board. The three standard limb leads and needle electrodes were employed. The experiment was made under ether anaesthesia. On five rabbits it was carried out in the following manner: The animal was anaesthetized and tied to the board, and the electrocardiographic tracings were subsequently recorded. The next step was to make a small incision medially to expose the gallbladder, which was grasped by aid of a forceps. The gallbladder was first emptied by suction with a hypodermic syringe of the ordinary type, the nozzle of which was inserted into the gallbladder and kept in position by tying it with silk. Then the gallbladder was gradually inflated to the capacity with air, care being taken not to make

TABLE 1

EFFECT OF DILATATION AND CONTRACTION OF THE GALLBLADDER UPON THE ELECTROCARDIOGRAM OF FIVE RABBITS

Frequency	Amplitude	QRS-T
Rabbit 1:		
ECG I: about 250/min.	nothing significant	no changes
ECG II: » »	» »	
ECG III: increased to about 300/min.	diminished in all Leads	
ECG IV: unchanged	unchanged	
ECG V: »	unchanged in Lead III, in Lead I as in ECG I	
Rabbit 2: (Fig. 1.)		
ECG I: about 350/min.	nothing significant	no changes
ECG II: » »	» »	» »
ECG III: » »	increased in Lead I, decreased in Leads II and III	SI increased, SII absent, QIII deepened and SIII decreased. TIII slightly negative
ECG IV: » »	unchanged	unchanged
ECG V: decreased to about 250/min.	»	»
Rabbit 3:		
ECG I: about 160/min.	nothing significant	no changes
ECG II: » »	» »	
ECG III: » »	» »	
ECG IV: » »	» »	
ECG V: increased to about 250/min.	decreased in Leads II and III	
Rabbit 4:		
ECG I: about 200/min.	nothing significant	no changes
ECG II: » »	» »	
ECG III: » »	» »	
ECG IV: » »	» »	
ECG V: increased to about 250/min.	decreased in Leads II and III	
Rabbit 5:		
ECG I: about 200/min.	nothing significant	no changes
ECG II: » »	» »	» »
ECG III: » »	» »	deepening of SII and SIII
ECG IV: » »	» »	unchanged
ECG V: » »	increased in Leads II and III	»

ECG I: under anaesthesia before operation.
ECG II: during contraction (Lead II).
ECG III: after contraction.
ECG IV: during dilatation (Lead II).
ECG V: after dilatation.

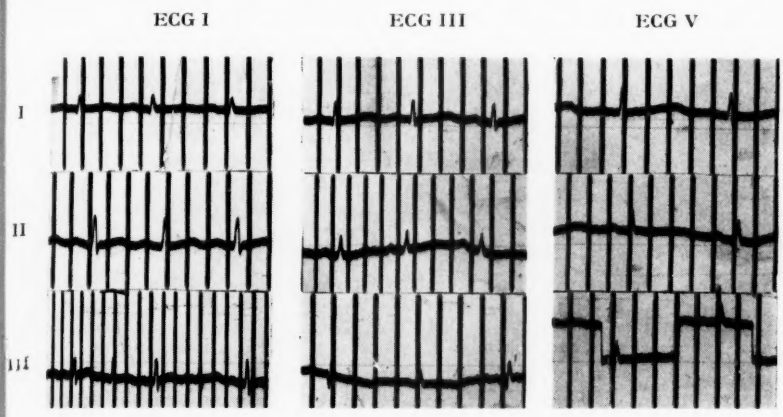

Fig. 1. — The effect of contraction and dilatation of the gallbladder upon the electrocardiogram of rabbit 2 of the first test series: ECG I: before the operation. ECG III: after the contraction. The amplitude is increased in Lead I, decreased in Leads II and III. SI increased, SII absent, QIII deepened anad SIII decreased. TIII slightly negative. ECG V: after the dilatation. The frequency decreased.

it burst. An electrocardiogram was taken upon each successive dilatation and contraction, as well as in the course of each procedure. The results are given in Table I.

On five additional rabbits the experiment was performed as follows: — Upon inserting into the gallbladder a cannula connected with a hypodermic syringe by means of a rubber catheter, the incised wound was closed up. The dilatation of the gallbladder was brought about by injecting it with saline or with air through the catheter, whereas the contraction was elicited by suction. The electrocardiographic records were taken under anaesthesia, both before the operation and immediately after it, next upon closing the abdomen, then both during the dilatation and the contraction of the gallbladder, and again after each of the two procedures. The results obtained were as follows:

Rabbit 1: (Fig. 2) Immediately after the operation the electrocardiogram showed lower oscillations in Leads II and III. In the subsequent electrocardiograms no further alterations were observed. The cannula was left in position. On the following day the experiment was repeated, both before and under anaesthesia, in the sequence stated. Even prior to the dilatation and contraction the electrocardiogram showed deeper SII and SIII than on the previous day. No alterations were produced by the dilatation and contraction processes themselves.

Rabbit 2: The procedure was the same as above. On the first day, there were no changes in the electrocardiogram. On the following day the experiment was repeated both before and under anaesthesia. No changes were recorded.

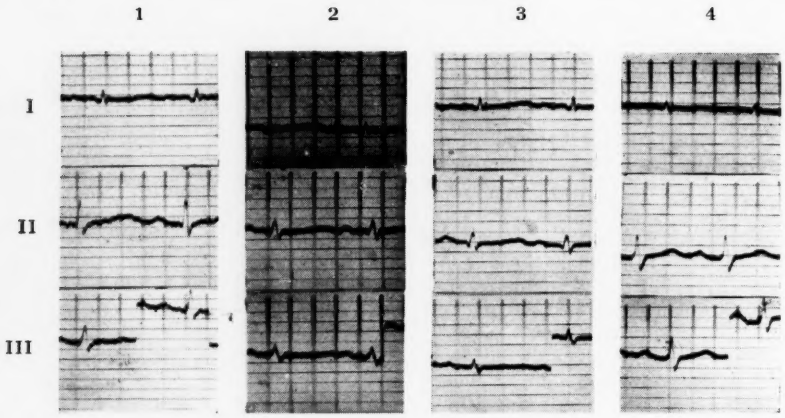

Fig. 2. — Rabbit 1 of the second test series: ECG 1: before the operation. ECG 2: immediately after the operation. Lower oscillations in Leads II and III. ECG 3: after the dilatation and contraction. No changes. ECG 4: on the following day before the dilatation and contraction. Deeper SII and SIII.

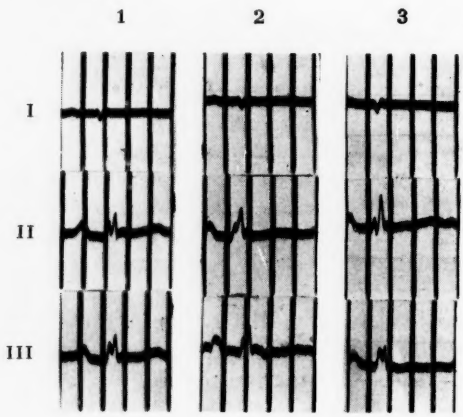

Fig. 3. — Cat 2 of the second test series: ECG 1: before the operation. ECG 2: immediately upon the operation. TII and TIII lowered. ECG 3: after the dilatation and contraction. No changes.

Rabbit 3: The procedure was the same as above. No changes were noted on the first day. On the following day each of the three leads of the electrocardiogram showed larger deflections than previously. No further changes were noted.

Rabbit 4: After inserting the cannula and closing the wound, the dilatation and contraction were delayed until the rabbit's awakening from the anaesthesia. No changes were recorded.

Rabbit 5: The procedure was the same. The deflections were smaller in leads II and III immediately after the operation. No changes were produced by the dilatation and contraction.

The same experiment was carried out on three cats. The results obtained were as follows:

Cat 1: After inserting the cannula and closing the wound the dilatation and contraction were delayed until the cat's awakening from the anaesthesia. These procedures produced no changes in the electrocardiogram.

Cat 2: (Fig. 3) The procedure was the same as above. TII and TIII were lowered immediately upon the operation, but maintained that level during the subsequent recordings. No further changes were noted.

Cat 3: The procedure was the same as above. No changes were recorded.

SUMMARY. — Ten rabbits and three cats were subjected to experimental dilatation and contraction of the gallbladder both under ether anaesthesia and in an unanaesthetized state. Electrocardiograms were taken simultaneously. The electrocardiographic tracings recorded no changes during the dilatation and contraction. After the operation and during the anaesthesia, various changes were noted in the amplitude and frequency of the deflections, and in the size of the various components of QRS. No constant nor characteristic changes suggesting pathological processes, particularly with reference to the shape of QRS-T, were observed.

The effect of inflammation of the gallbladder upon the electrocardiogram was studied in the following manner: — The gallbladder of an anaesthetized rabbit was injected with colisuspension in broth by means of an ordinary hypodermic syringe and the site of injection ligated with catgut. Both prior to the operation and immediately after the injection, an electrocardiogram was taken of the animal under anaesthesia. Repeated electrocardiograms were taken at intervals of a few days. The results were as follows:

Rabbit 1: The ECG preceding and following the operation showed nothing significant. After twenty days the ECG was taken again, but showed no alterations. Later on the rabbit succumbed. There was no post-mortem examination.

Rabbit 2: The ECG taken prior to and after the operation showed nothing significant. Two days later a new ECG was taken, but showed no changes. Subsequently the rabbit succumbed. There was no post-mortem examination.

Rabbit 3: The same as above: no changes.

Rabbit 4: Nothing to be noted in the ECG preceding and following the operation. After three days another ECG was taken: TIII was negative. Six days later TIII was less negative. Twenty days later TIII

was small but positive. The rabbit succumbed subsequently. Post-mortem examination revealed numerous adherences in the abdominal cavity and an atrophied, thick-walled, suppurating gallbladder.

Rabbit 5: Nothing remarkable was found in the ECG taken before and after the operation. Three days later the oscillations in all leads were somewhat smaller than previously. On the following day the rabbit succumbed. Post-mortem findings: inflammation of the galibladder and its surroundings. The gallbladder was atrophied and filled with heavy pus.

Rabbit 6: The ECG taken before and after the operation showed nothing significant. On the subsequent day the rabbit succumbed. Post-mortem examination revealed an atrophied gallbladder containing heavy bile.

Rabbit 7: The ECG taken prior to and after the operation showed nothing significant. Two days later the rabbit succumbed. The post-mortem finding was a gallbladder filled with heavy bile.

Rabbit 8: Nothing to be noted in the ECG. A few days later the rabbit succumbed. The was no post-mortem examination.

Rabbit 9: Nothing significant in the ECG before nor after the operation. On the subsequent day the rabbit succumbed. No post-mortem examination was made.

Rabbit 10: The ECG before and after the operation revealed nothing significant. Twenty days later another ECG showed that the oscillations of Lead I and II were larger than previously. No further alterations were noted. The rabbit succumbed later on. No post-mortem examination was made.

The experiment was also carried out on four rabbits in the following manner: In addition to the injection with coli-bacteria, the gallbladder was, in three cases, filled with some twenty glass beads of 1½ mm in diameter, whereas in one case a single bead of 5 mm in diameter was inserted. The results obtained were as follows:

Rabbit 1: The ECG taken before and after the operation revealed nothing significant. Three days later SII and SIII were somewhat deeper than in the previous records. In each of the three leads the oscillations were five days later larger than previously. SII and SIII showed further deepening. Twelve days later the ECG was the same as in the beginning.

Rabbit 2: The ECG taken before and after the operation revealed: nothing significant. Three days later the deflections in each of the three leads were smaller than in the previous tracings. Five days later the deflections in Lead I were larger, in Lead III somewhat smaller than in the previous ECG. TII was isoelectric, TIII slightly negative. Twelve days later RI was larger, QII deeper than previously, TI isoelectric, SII absent. A few days later the rabbit succumbed. Post-mortem examination revealed suppuration of the operative wound, two liver abscesses the size of a thumb-nail, containing heavy pus. The gallbladder was thickwalled, filled with heavy pus and glass beads.

Rabbit 3: The ECG recorded before and after the operation revealed nothing significant. Three days later the deflections in Lead III showed largening. Five days later TIII was negative. Twelve days later the deflections in Leads II and III were larger than in the previous records.

Rabbit 4: (a single large bead) The ECG before and after the operation revealed nothing significant. On the following day no changes were noted in the ECG. Ten days later there were no alterations. The rabbit succumbed subsequently. No post-mortem examination was made.

SUMMARY. — The gallbladders of ten rabbits were injected with coli-bacteria. Glass beads have been inserted into the gallbladder of four other rabbits. Electrocardiographic tracings were recorded both prior to the operation and after it. The recording was repeated at varying intervals. In one of the rabbits TIII showed a transient shift to the negative. Those rabbits which were specially prepared by inserting glass beads into the gallbladder showed various alterations in the amplitude of the deflections and in the size of the components of QRS, but no constant nor characteristic changes.

COMMENT

Dilatation and contraction of the gallbladder was carried out on ten rabbits and three cats, both under anaesthesia and without it. These procedures produced in the electrocardiogram various alterations in frequency, either increase or decrease, as well as changes in the amplitude of the deflections and the various components of QRS. The surgical shock and the effect of prolonged anaesthesia (SCHAFER, UNDERWOOD and GAYNOR 1929) might suffice to explain the varying electrocardiographic changes. Not one of the electrocardiograms, it should be noticed, showed changes suggestive of distinct contraction of the coronary arteries. On ten rabbits of the latter test series the gallbladder was injected with coli-bacteria. Glass beads were inserted into the gallbladder of the remaining four rabbits. Nor did the electrocardiographic tracings of these animals yield signs indicative of contraction of the coronary arteries due to the inflamed gallbladder. It is to be noted that a large proportion of these rabbits developed a fatal septic state without presenting appreciable alterations in the electrocardiogram. From the above experiments, consequently, it cannot be concluded that the reported animals actually displayed a reflex which, referred from the irritated gallbladder into the cardiac region, caused contraction of the coronary vessels.

SUMMARY

Dilatation and contraction of the gallbladder in rabbits and cats as well as experimentally induced cholecystitis + cholelithiasis in rabbits did not produce in the three standard limb leads of the electrocardiogram any constant, characteristic deviations from the normal.

REFERENCES

Babcock, R. H.: J. A. M. A. 1909:*52:*1904.
Bean, W. B.: Amer. Heart. J. 1937:*14:*684.
Bellet, S.: Tri-State Med. J. 1938:*11:*2177.
Bettman, B. B., & Rubinfeld, S. H.: Amer. Heart J. 1935:*10:*550.
Boyd, A.: Amer. J. Path. 1928:*4:*159.
Breyfogle, H. S.: J. A. M. A. 1940:*114:*1434.
Brockbank, E. M.: Edinbgh. Med. J. 1898:*4:*51.
Buchbinder, W. C.: Proc. Soc. Exper. Biol. a. Med. 1930:*27:*542.
Campbell, S. B.: Brit. Med. J. 1936:*1:*781.
Clarke, N. E.: Amer. Heart J. 1945:*29:*628.
Constancinescu, M. N., & Tuchel, V.: Zbl. Chir. 1939:*66:*104.
Danielopolu: Quoted by Constancinescu and Tuchel, ibidem.
Donoso, J., Donoso, & Steiner: Rev. Méd. Chile 1946:*74:*515.
Eckenhoff, Hafkenschiel, & Landmesser: Amer. J. Physiol. 1947: *148:*582.
Hinrichsen, & Ivy: Arch. int. Med. 1933:*51:*932.
Hodge, G. B., Messer, A. L. & Hill, H.: Arch. Surg. 1947:*55:*710.
Laird, S. M.: Brit. Med. J. 1938:*1:*884.
McArthur, S. W., & Wakefield, H.: Proc. Central. Soc. Clin. Research 1944:*17:*7.
Miller, C. H.: Lancet 1932:*1:*767.
Rolleston, D.: Brit. Med. J. 1920:*1:*319.
Schafer, G. D., Underwood, F. J. & Gaynor, E. P.: Amer. J. Physiol. 1929:*91:*416.
Schwartz, M., & Herman, A.: Ann. Int. Med. 1931:*4:*783.
Taquini, H. C., Yodice, C. & Taquini, A. C.: Rev. argent. Card. 1947:*14:* 117.
Tennant, R., & Zimmerman, H. M.: Yale J. Biol. a. Med. 1931:*3:*495.
Vest, V. E.: South. med. J. 1934:*27:*410.
Wilhelmy, E. W., & Helwig, F. C.: J. Missouri Med. Assoc. 1935:*12:*476.
Willius, F. A., & Brown, G. E.: Amer. J. Med. Sci. 1924:*168:*165.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

BLOOD CULTURES IN THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

By

M. E. PARMALA

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Blood cultures give conclusive results, provided the contamination percentage is reduced to a minimum and growth occurs. In spite of most favourable conditions when taking, sending, and cultivating the samples, the results can often be negative, even in a fairly high percentage of cases. Table I illustrates the results obtained elsewhere.

TABLE I

GROWTH OF PATHOGENIC BACTERIA ACCORDING TO DIFFERENT STATISTICS

Authority	Year	Patients	Cultures	Pathog. Bacteria	Pathog. Bact. per cent
Lichtman	1932	3869	5233	762	14.6
Jörgensen	1936		93	22	23.7
Dulaney	1937		175	35	20.0
Fox	1940	3423*	5310		20.6
Mollov	1942		579		17.5
Shearer	1945				30.0
Reynes	1947		8620	2362	27.4
Own Results	1949		2103	279	13.3

Among the cases described by Lichtman (4) there are many where pathogenic bacteria had grown unexpectedly. In cases of endocarditis lenta growth occurred in 95 per cent.

Jörgensen's (3) statistics only contain sepsis, endocarditis, and rheumatic fever, whereas pneumonia, typhus, etc. have not been included. The large number of positive results is due, in his opinion,

to the use of the anaerobe method parallel with the one generally employed; the former produced a growth of several streptococcus strains, while the aerobe cultures remained sterile.

Among the cases reported by Dulaney (1) typhoid fever is relatively abundant (13 cases), which increases the percentage of positive results.

In the statistics compiled by Fox (2) the percentage of growth is, according to himself, too great and therefore missleading, owing to high values for typhoid fever (58 per cent) and also for endocarditis lenta, pneumonia, and surgical sepsis.

Mollov (5) has taken all growth into account, without any differentiation of pathogenity. By using the classification I have employed (page 171) the positive results ought to be reduced by 4.7 per cent.

In Reynes' (8) statistics the percentage of growth was increased when the anaerobe method was used, augmenting the number of positive results by 94 (4 per cent).

Shearer's (9) growth percentage is high but among the positives are also counted *Proteus vulgaris*, as well as *Escherichia coli*, which are generally considered contaminants. He could, by improving the technique, reduce the contamination percentage of about 50 even to 13.8 per cent.

In the course of the last five years our laboratory received in all 2103 blood specimens, on which blood cultures were performed. Part of the samples came from the country as citrated blood and were inoculated into culture tubes at the laboratory, while the majority were already inoculated into tubes supplied by the same laboratory. As nutrient medium the laboratory generally used 4 per cent peptone water with 0.2 per cent glucose with a pH of 7.4 up to November 1946 and since then a 1 per cent buffered beef peptone with 0.1 per cent glucose and pH 7.4. To bottles containing 45 ml of culture medium 10 ml of blood have been added in some cases and in others the blood has been divided into two bottles containing 3 ml and 7 ml respectively.

The number of specimens has steadily increased with each year. Growth ensued in 649 (30.9 per cent), but bacteria to be regarded as »pathogenic» only grew in 279 (13.3 per cent) instances. The incidence and classification of different bacteria is illustrated by Table II. During this five year period fully negative cultures

TABLE II

BACTERIA ISOLATED FROM BLOOD CULTURES

Pathogenig	Number
Streptococcus viridans	56
» pyogenes	24
» nonhaemol.	20
» anaerobicus	4
» lanceolatus	1
» faecalis	3
» atypica (weak virulens)	4
Diplococcus pneumoniae	2
Haemophilus species?	2
Gr- diplococcus	1
Staphylococcus aureus	105
» albus haemolyticus	5
Salmonella typhi	11
» paratyphi B	41
Non-pathogenic	
Staphylococcus albus	170
» citreus	2
Escherichia coli	31
Bacterium paracoli	26
» faecalis alcaligenes	98
Gr- rod	6
Proteus vulgaris	6
Pseudomonas aeruginosa	14
Corynebacterium pseudodiphteria	6
Bacterium subtilis	10
Gr + rod	9
Sarcina	1
Neisseria	3
Micrococcus antibioticus	1
Yeast fungus	2
»Contaminated»	26

totalled 1405 (67.3 per cent) and 39 cases (1.3 per cent) had to be discounted owing to technical errors and accidents. There has been a regular yearly decrease in contaminated samples, their number having decreased in the course of last year to 12 per cent, i.e., below the limit (14 per cent) required by Shearer (9).

Diagnosis was available for 697 different patients. The cases were divided[1] on the basis of the diagnosis into three groups: into those where according to the diagnosis bacteremia was not to be expected, into those where bacteremia was possible but

[1] In the classification of the cases I have received valuable assistance from Dr. P. I. Halonen, to whom my best thanks are due.

TABLE III

DIVISION OF THE CASES INTO DIFFERENT GROUPS ON THE BASIS OF DIAGNOSIS, TAKING INTO ACCOUNT THE POSSIBILITY OF BACTEREMIA

Group I	Noninfective surgical diseases (e.g. prolapsus recti)	13
	Various tumors	17
	Gastro-intestinal diseases	13
	Blood diseases	29
	Cardiovascular diseases	16
	Local infections, with no bacteremia	10
	Tuberculous processes	25
	Diseases caused by virus, fungi, or parasites	17
	Tegumentary, allergical, endocrine a.o. misc. diseases	48
	Total	188
Group II	Rheumatic fever	32
	Acute and rheumatic endocarditis	36
	Infectio acuta	41
	Febris e causa ignota	15
	Nephritis acuta	10
	Gastroenteritis acuta	35
	Surgical infections, probably with bacteremia (cholecystitis purulenta, etc.)	13
	Gynecological and other infections, there may be bacteremia (endometritis, etc.)	49
	Subchronic and chronic infections	5
	Total	247
Group III	Endocarditis lenta	61
	Sepsis	54
	Pneumonia	45
	Typhoid fever and Salmonella fever	34
	Surgical infections (phlegmon etc.)	44
	Abortus febrilis	5
	Meningitis purul., otitis media a.o. misc. infections	19
	Total	262

not permanent, the third group consisting of cases in which the disease, at least at some stage of it, was regularly associated with bacteremia. The grouping of the cases is presented in Table III.

Table IV (and V) illustrates the results of blood cultures in these different groups. We note that bacteria generally regarded as pathogenic also grew in the cultures belonging to the first group. The growth of pathogenic bacteria is most frequent in the third group.

TABLE IV

GROWTH IN DIFFERENT DIAGNOSTIC GROUPS

	I		II		III		Total	
	No.	%	No.	%	No.	%	No.	%
Specimens	188	(27.0)	247	(35.4)	262	(37.6)	697	
Pathog. bact. ..	12	*6*	24	*10*	64	*24*	100	*14.3*
Contaminated ..	27	14	47	19	51	19	125	17.9
Negative	149	79	176	71	147	56	472	67.8

TABLE V

RESULT OF TREATMENT IN DIAGNOSTIC GROUPS

Treatment (Total)	I			II			III			Total No. of Cases
	Cases	Path. Bact.		Cases	Path. Bact.		Cases	Path. Bact.		
		No.	%		No.	%		No.	%	
Pen. >250.000 un.	15	2	13	28	3	11	35	3	9	78
Pen. <250.000 un.	8	2	25	17	1	6	17	4	24	42
Var. sulphadrugs	6	—	—	23	3	13	25	—	—	54
Streptomycin	—	—	—	1	—	—	1	—	—	2
treated cases, total ..	29	4	14	69	7	10	78	7	9	176
untreated cases	64	1	1	97	5	5	65	8	12	226
Total No. of Cases	93	5	5	166	12	7	143	15	10	402

The records available for 402 patients contained information on the therapy. As evidenced by Table V, about one-half of the patients were given some kind of antibacterial treatment before the blood cultures were made, about one fifth of them having even received significant amounts of penicillin. In spite of large doses of penicillin, pathogenic bacteria sometimes continued to grow in the blood cultures. The growth was surprisingly frequent in these cases, and still more frequent in group one than in groups two and three.

In cases of endocarditis lenta, 18 per cent exhibited a growth of *Streptococcus viridans* and 10 per cent some other pathogenic micro-organism, such as anhemolytic or anaerobic streptococcus, *Staph. aureus* or *Haemophilus*, which may have caused endocarditis. In 21 per cent the patient was administered penicillin prior to the

taking of the sample, which may have been responsible for the low percentage of growth.

In sepsis, pathogenic bacteria had grown in 23 per cent. Penicillin medication possibly inhibiting the growth had been given prior to the taking of the specimen in 13 per cent. 3 cases exhibited a growth of *Pseudomonas aeruginosa*. One of them showed symptoms of endocarditis lenta and a repeated growth of *Pseudomonas*. The remaining two were children also affected with gastroenteritis, death ensuing in both cases. In neither case had blood cultures been made more than once.

Of the 29 Salmonella fever patients 7 had a history of more than two weeks' fever. Of the remaining 22 growth ensued in 59 per cent.

No growth could be produced in a single instance of postabortal febrile cases, probably due to the circumstance that the laboratory has not generally made use of the anaerobe method in routine proceedings.

TABLE VI

GROWTH OF STAPHYLOCOCCUS AUREUS, STAPHYLOCOCCUS ALBUS AND COLIFORM BACTERIA IN DIAGNOSTIC GROUPS

	I		II		III	
	Number	%	Number	%	Number	%
Staph. aureus	4	2	8	3	18	7
Staph. albus	12	6	19	8	33	13
Coliforms	9	5	21	9	33	13

Table VI illustrates the growth of *Staphylococcus aureus*, *Staphylococcus albus* and coliform bacteria in the different diagnostic groups. As we see, the growth percentages increase proportionately for every bacterium.

DISCUSSION

The results of blood cultures obtained at the Department are not as good as those obtained elsewhere (Table I). This may be partly due — as illustrated by Tables III and IV — to the relatively large proportion (about 30 per cent) of cases where bacteremia was not to be expected. The extensive antibacterial

treatment administered immediately before the taking of the cultures also contributed to the keeping down of the percentage of positive cultures. Yet our very simple nutrient substance might also be considered as a factor reducing the number of positive results. As far as last year's results are concerned, the contamination percentage can be regarded as lying on a satisfactory although not optimal level.

It is rather interesting to see how frequent are the cases of asymptomatic bacteremia in my statistics. It is possible (7) to obtain bacterial growth in the blood of healthy persons. It may partly explain the results in the cases with unexpected growth. There might also have been cases with a »vague diagnosis», as, for instance, in the first group one third had received antibacterial treatment (Table V) and therefore the suspicion must be entertained that the patient might have exhibited some signs indicative of infection even if the diagnosis gave not evidence of it.

Antibacterial treatment administered immediately before the taking of blood specimens prevents a positive result in the majority of cases. But if severe local infection is involved, continually discharging bacteria into the blood, growth can be brought about. This is illustrated by a patient whose cultures, notwithstanding the administration of several millions of units of penicillin, showed a persistent growth of *Staphylococcus aureus*. The diagnosis was septicaemia and pneumonia, to which endocarditis ulcero-verrucosa was added on the basis of the autopsy. In complicated cases a positive blood culture in spite of abundant antibacterial medication can therefore have a prognostic value.

The results obtained in cases of endocarditis lenta and particularly in sepsis cannot be considered satisfactory, when compareing them for instance to corresponding results obtained by Lichtman (4). The media used by us seem not to be good enough.

The growth of *Pseudomonas aeruginosa* should not be regarded always as due to contamination. DeMuth (6) describes a case which began with abdominal symptoms and led to death from endocarditis. *Pseudomonas* grew repeatedly in the blood culture, and the bacterium could also be made to grow in specimens taken postmortally. Autopsy revealed massive endocarditis as well as considerable changes in other organs. It was ascertained that *Pseudomonas aeruginosa* had invaded the circulation from abdo-

minal organs, which is also supported by the symptoms at the onset. Referring to this case, he recommends, whenever a growth of *Pseudomonas* is demonstrable, checking it by new cultures, and if the positive results persist, to suspect *Pseudomonas aeruginosa* as the causative agent. The three cases previously mentioned give rise to the suspicion that they are of the same nature as the one just described.

Especially interesting results are seen in Table VI. The growth of *Staphylococcus albus* and Coliforms are generally considered contaminant. According to the table where the growth percentages are rising from the first to the third group in the same way as percentages of *Staphylococcus aureus* which can be considered pathogenic, we can find that among the cases of *Staphylococcus albus* and Coliform growth, there may be also true bacteremia, all the more because *Staphylococcus albus* grew in many cases of osteomyelitis and Coliforms — in many cases of gastroenteritis and gluteal or some other pelvic abscess or phlegmon. The contamination cannot be variable according to the diagnosis. It would be particularly interesting to verify the results in larger statistics elsewhere.

SUMMARY

Of the 2103 specimens examined 279 (13.3 per cent) produced a growth of pathogenic bacteria.

Contamination has steadily decreased, amounting last year to 12 per cent.

A growth of pathogenic bacteria was also demonstrated in such cases, where, according to the diagnosis, bacteremia was not to be expected.

An antibacterial treatment administered to the patient does not entirely eliminate the serviceableness of blood cultures, on the contrary, they can be of prognostic value and, in consequence of the limits set up by specificity, also possess a diagnostic significance.

The growth percentages of *Staphylococcus albus* and Coliforms in diagnostic groups are rising in the same way as the growth percentages of *Staphylococcus aureus*.

REFERENCES

1. Dulaney, A. D., and Guthrie, F.: J. Lab. Clin. Med. 1937:*22:*721.
2. Fox, H. and Forrester, J.: Am. J. Clin. Path. 1940:*10:*493.
3. Jörgensen, J. V.: Hospitalst. 1936:*79:*611.
4. Lichtman, S. S., and Gross, L.: Arch. Int. Med. 1932:*49:*1078.
5. Mollov, M., Winter, J., and Steinberg, Ph.: Am. J. Clin. Path. 1942:*12:*571.
6. DeMuth, W. E. Jr, and Rawson, A. J.: Am. J. Med. Sc. 1948:*216:*195.
7. Reith, A. F., and Squier, T. L.: J. Inf. Dis. 1932:*51:*336.
8. Reynes, V., et Prevot, A. R.: C. R. Soc. Biol. 1947:*141:*261.
9. Shearer, Ch.: Edinburgh Med. J.: 1945:*52:*420.

AUS DEM SERO-BAKTERIOLOGISCHEN INSTITUT DER UNIVERSITÄT HELSINKI

ÜBER DAS VORKOMMEN VON TETANUS NACH KRIMINELLEM ABORT

Von

TAPIO SAVOLAINEN

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Der Starrkrampf gehört zweifellos zu den seltenen puerperalen Infektionen, es gibt aber zahlreiche Untersuchungen darüber, die im ganzen ca. 200 Fälle behandeln. Dagegen ist der Tetanus nach kriminellem Abort bedeutend seltener, obgleich die Infektionsmöglichkeiten hier offensichtlich grösser sind. In einem Teile der Fälle kann natürlich der septische Zustand vor der Entwicklung des Starrkrampfes zum Tode geführt haben, und alle Fälle sind ja wohl kaum veröffentlicht worden.

Von der Natur des kriminellen Abortes hängt es ab, dass ausser denjenigen Fällen, wo das verbrecherische Verfahren zugegeben oder festgestellt worden ist, es eine Anzahl von Aborten desselben Ursprungs gibt, wo dieser sich aus irgendeinem Grunde nicht mit genügender Klarheit ermitteln liess. Somit muss man damit rechnen, dass es unter solchen Fällen, wo der Starrkrampf nach einem als spontan angegebenen Aborte entstanden war, auch unbestätigte kriminelle Aborte gibt.

Über die Entwicklung des Tetanus nach Abort gibt es viele Untersuchungen, u.a. von HEINRICIUS (5) 1 einheimischer Fall, VINAY (27) 47, MEINERT (11) 1, ROSE (24) 4, STEINITZ (21) 1, FLATAU (2) 2, KURTTIO (8) 1, und MIRONESCU u. BALS (22) 2 Fälle.

Der erste Fall von Starrkrampf nach sicher krimineller Fruchtabtreibung wird in SATTLERS (15) Abhandlung vom J. 1890 behandelt. Danach sind jedenfalls noch folgende Fälle veröffentlicht worden: SEEGERT (18) 1, SCHOTTMÜLLER (17) 2, Kraus (6) 1, FREUND (4) 2,

SPIEGEL (20) 1, ROTHSCHILD (14) 2, SIMON (19) 3, PROST (12) 1, SCHNEIDER (16) 2, VÖLCKER (28) 1, und FLECHTNER u. QUAST (3) 2 Fälle.

Zu beachten ist die geringe Anzahl der veröffentlichten Tetanusfälle nach Abort, obgleich der Starrkrampf z. B. in den Tropen eine ziemlich häufige Todesursache darstellt: nach WARING (26) starben von 42,651 Toten 912 an Tetanus, und davon 232 an puerperalem Tetanus.

Die Erforschung der Infektionsquelle ist von besonderem Interesse. Die Infektion hat offensichtlich durch Personen herbeigeführt werden können, welche innere Untersuchungen anstellten (5), oder hat sie sich die Patientin bei Gartenarbeiten zuziehen können (21). Andere Forscher halten die Gedärme für die Infektionsquelle (4), sind doch in den Gedärmen Tetanusbazillen oder ihre Sporen recht reichlich vorhanden, nach TENBROECK und BAUER (23) 34.7 %, nach BUZELLO und RAHMEL (1) 40 %. Die Letzteren stellten fest, dass Personen mit Karzinom oder ulzerösen Prozessen im Magen-Darmkanal, ganz besonders viel Tetanusbazillen hatten. Dagegen gehört der Starrkrampfbazillus nicht zur vaginalen Flora, was MARTIN (10) bewiesen hat, indem er mit dem Vaginalsekret von 100 Patientinnen, ohne besondere Auswahl, die Mäuseprobe anstellte. In keinem einzigen Falle liessen sich Tetanusbazillen feststellen. In den 3 von MEINERT (11) veröffentlichten Fällen war die Ursache der Infektion offensichtlich der Bozemannsche Uteruskatheter, der bei der Behandlung sämtlicher Fälle gebraucht wurde. Eine besondere Infektionsquelle lag auch in FLATAUS (2) beiden Fällen vor, wo eine Uterussonde gebraucht wurde, an dessen Lackanstrich Tetanusbazillen gefunden werden konnten.

Das Verfahren bei manchen kriminellen Aborten verbleibt natürlich in Dunkel verhüllt; sonst pflegt es sehr verschieden zu sein. Zuweilen hat die Patientin sich selbst mit einem dünnen Holzstöckchen in den Uterus gestochen, eine behauptete es mehrmals getan zu haben (3), zuweilen haben die Patientin selbst oder der Abtreiber, darunter sogar Ärzte, Seifenwasser u.a. in die Gebärmutter gespritzt. Auch Vaginalspülungen sind gemacht worden. Im Falle KRAUS (6) wurde eine Wurzel der Malva communis gebraucht, an der es später Tetanusbazillen zu finden gelang.

Die Tetanusinfektion bei kriminellem Abort ist offensichtlich durch äussere Eingriffe in den Uterus oder in dessen lädierte Umgebung verursacht worden.

Obgleich es unter den obenerwähnten Fällen einige Tetanusmonoinfektionen gegeben hat, ist gemischte Infektion als Regel zu betrachten, da ja solche Zustände bei septischem Abort vorherrschen. Auf experimentellem Wege hat es sich dagegen nachweisen lassen, dass keine Tetanuserkrankung entsteht, wenn man Versuchstieren Starrkrampfbazillen injiziert, aus denen das Toxin entfernt worden ist (25).

Die Inkubationsperiode scheint bei puerperalem Tetanus etwas kürzer (2—11 Tage) als bei traumatischem zu sein, wo sie oft über 12 Tage beträgt (3). In ca. einem drittel der von KURTTIO (8) beschriebenen Fälle hat die Inkubationsperiode über 12 Tage gedauert.

Als erste Symptome treten regelmässig Beschwerden beim Schlucken oder Halsschmerzen und Steifheit im Unterkiefer auf, worauf sich ziemlich regelmässig das vollständige klinische Bild des Tetanus zu entwickeln pflegt.

Die Resultate der Behandlung sind äusserst schlecht (Sterblichkeit 85—95 %), schlechter als beim traumatischen Tetanus, wo die Sterblichkeit nach KURTTIO (8) durchschnittlich 61 % betraf. Das liesse sich teils dadurch erklären, dass während der Schwangerschaft im Uterus vorzügliche Resorptionzustände herrschen und anderseits die Wege bis zum Rückenmark kurz sind.

Beruhigende Mittel, Serumbehandlung und eine radikale Uterusamputation haben nur in seltenen Fällen Resultate gezeitigt und auch nur dann, wenn Serumbehandlung und Amputation gleichzeitig unternommen wurden. Nur MIRONESCU und BALS (22) melden die Genesung zweier Tetanuspatientinnen nach Abort durch Serumbehandlung, wobei enorme Mengen Serum, d.h. über 3,000,000 Einheiten, oder 6 Liter per Patientin verabfolgt wurden.

Bakteriologische Untersuchungen sind in den veröffentlichten Fällen relativ wenig unternommen worden. Gewöhnlich hat man sich mit der Feststellung der Tetanusbazillen durch Tierproben begnügt, aber auch das ist nicht immer geglückt. So ist im Falle SEEGERT (18) die Tierprobe bei der Verwendung des der lebenden Patientin entnommenen Vaginalschleimes gelungen, nicht aber bei der Obduktion. Auch in SCHOTTMÜLLERS (17), SPIEGELS (20) und FLECHTNERS u. QUASTS (3) Fällen gelang der Nachweis der Tetanusbazillen. Bei anderen wiederum, wie bei PROST (12), ergaben sowohl die bakteriologische Untersuchung, als auch die Tierprobe

ein negatives Resultat. In anderen Fällen, wie bei SCHNEIDER (16) und VÖLCKER (28), entsprach dem klinischen Tetanus ein bakteriologischer Gasbrand. In diesen Fällen handelte es sich ohne Zweifel um Starrkrampf, und Clostridium Welchii trat als Nebenfund auf. Das bestätigen auch die Untersuchungen von LEHMAN und FRAENKEL (9), denen es gelang im Cervicalsekret von 580 untersuchten Abortfällen bei 106 Patientinnen Cl. Welchii ohne klinische Symptome zu finden.

In meinem Falle habe ich bakteriologische Untersuchungen angestellt, aber keine vollständige Anamnese erhalten können. Gemeinsam mit dem von KURTTIO (8), beschriebenen Falle ist der gleiche Erkrankungsort.

E. L. B. 38-jährig, Arbeiterin aus Pietarsaari, besuchte den Arzt am 3. 4.—48 und 9. 4.—48, wobei Gravidität im frühestens dritten Monat festgestellt wurde. Als der Arzt den erwünschten Abort nicht ausführen wollte, sagte Pat., sie würde ihn anderweitig machen lassen. Am 3.5.—48 wurde ein anderer Arzt zur Patientin ins Haus gerufen, der Kieferstarre feststellte und Pat. ins Krankenhaus brachte. Trotz Serum und Medizinen starb Pat. am 5. 5.—48, wobei das klinische Krankheitsbild am nächsten einem fiebrigen Starrkrampf entsprach. Die am 12. 5.—48 unternommene (gerichtsmedizinische) Obduktion zeigte keine äusseren Verletzungen. In den inneren Organen ausser dem unten Angeführten nichts besonderes. Die äusseren Geburtsteile waren etwas angeschwollen, Uterus $3 \times 10 \times 13.5$ cm, seine Mündung klaffte 4 cm weit auf, war dunkelrot, enthielt Placentastücke und geronnenes Blut; Milz $11 \times 9 \times 3$ cm, schlaff, Oberfläche glatt, feucht, innere Struktur unklar.

Zwecks bakteriologischer Untersuchung wurde Blut in ein steriles Röhrchen, wie auch ein Teil der Milz und die Hälfte des Uterus in ein und dasselbe Glasgefäss entnommen.

Die Abtreiberin wurde wegen Abtreibung und fahrlässiger Tötung gerichtlich belangt.

Die bakteriologischen Untersuchungen begannen am 15. 5.—48; es wurde ZEISSLERS (29) Vakuummethode verwendet, als Nährboden dienten Glykoseblutagarschalen und Leberbrühe- und halbflüssige Agar- und halbflüssige Glykoseagarröhren. Von allen Proben wurden direkte Kulturen und solche nach fraktionierter Erhitzung auf + 100° C gemacht. Die Zeit des Erhitzens schwankte von einigen bis 15 Minuten.

Nach 3 Tagen bemerkte man bei den direkten Kulturen Grampositive Gruppen- und Kettenkokken, wie auch grosse Gram-positive Stäbchen, die sich später als aërobe, eventuell zur Vaginal-

flora gehörende Bakterien erwiesen. Ausserdem fand man längliche, dünne Stäbchen, welche gleich den Tetanusbazillen terminal Sporen bildeten. Die Sporen waren rund und bedeutend dicker als die Stäbchen. Bei den Kulturen aus erhitzten Proben war der Befund zum Teil der gleiche, zum Teil nur ein Bruchteil desselben.

Mit halbflüssigen Agarkulturen wurden an 2 Meerschweinchenpaaren folgende Proben gemacht: vom einem Paar erhielt jedes Tier à 0.5 cm^3 einer 3 Tage alten Kultur, die morphologisch Tetanusbazillen ähnliche Stäbchen enthielt. Ein Tier ausserdem 600 Einheiten, oder 1 cm^3 Tetanusantitoxin. Mit dem anderen Meerschweinchenpaar wurde dieselbe Probe gemacht, nachdem dieselbe Kultur 10 Minuten lang durch heisses Wasser auf + 80° C erwärmt worden war. Injiziert wurde intraperitoneal.

Die Versuchstiere des ersten Paares Starben beide nach 26 Stunden, ohne dass Tetanussymptome bemerkt wurden. Offenbar war der Tod durch andere in der Kultur enthaltene Bakterien, zunächst durch Strepto- und Staphylokokken, verursacht worden.

Vom zweiten Meerschweinchenpaar starb das eine nach 46 Stunden, während das Meerschweinchen, welches Antitoxin erhalten hatte, noch nach 1 Monat ganz munter war.

Durch weitere Kultivierung von erhitzten halbflüssigen Agarkulturen gelangte man zur reinen Kultur, deren Stäbchen, die nach 48 und 72 Stunden Sporen bildeten, morphologisch vollständig dem Cl. Tetani entsprachen. Mit dieser Kultur wurden weitere Versuche an einem Mäusepaar unternommen. Zunächst erhielt jedes Tiere 0.1 cm^3 einer 3 Tage alten Kultur, eines davon ausserdem 0.1 cm^3 oder 60 Einheiten Tetanus-antitoxin. Schon nach 2 Stunden erkrankte eine Maus und starb 16 Stunden später an Krämpfen. Diejenige Maus, die Antitoxin erhalten hatte, war 6 Tage lang munter, starb aber dann am siebenten Tage, allerdings ohne Krämpfe. Dieser Versuch wurde an drei weiteren Mäusepaaren wiederholt. 3 Mäuse starben nach 21 Stunden an Krämpfen, während diejenigen, die Antitoxin bekommen hatten, noch nach einer Woche ganz munter waren.

Die obenerwähnten Tierversuche wurden mit Kulturen aus der Milzprobe gemacht. Da die Milz und der Uterus in ein und demselben Glasgefäss verschickt worden waren, kann man natürlich nicht mit Sicherheit sagen, ob erstere schon beim Absenden Tetanusbazillen enthielt, aber es ist sehr wahrscheinlich, da auch in der

Blutprobe Bazillen vom Tetanustypus gefunden wurden. Im Obduktionsmaterial haben REINHARDT u. ASSIM (13), und KÜHNAU (7) schon früher Tetanusbazillen u.a. im Blut, in der Leber, der Milz, den Nieren und den Lymphdrüsen festgestellt.

Obgleich in dem oben beschriebenen Falle die Obduktion erst 7 Tage nach dem Tode ausgeführt wurde, handelt es sich meiner Ansicht nach offensichtlich um einen durch exogene Tetanusinfektion verursachten letalen Ausgang. Dies bestätigen übereinstimmend sowohl das klinische Krankheitsbild als die bakteriologische Untersuchung.

RÉSUMÉ

Ayant étudié environ 20 cas d'avortement criminel suivi du tétanos mentionnés dans la littérature médicale, l'auteur donne un bref aperçu des procédés d'avortements criminels et de l'infection qui s'en suit, dresse le tableau clinique du tétanos et traite des méthodes et des résultats du traitement. Ensuite il présente un cas d'avortement criminel suivi du tétanos à issue mortelle, où l'on a pu, en employant la méthode ZEISSLER, constater les bacilles de tétanos dans le sang, dans la rate et dans la matrice, démontrer qu'ils sont pathogéniques pour les cobayes et les souris, et les obtenir en culture pure.

SCHRIFTTUM

1. BUZELLO, A., und RAHMEL, O.: Arch. klin. Chir. 1924:*130:*660.
2. FLATAU, S.: Refer. nach SCHNEIDER.
3. FLECHTNER, H., und QUAST, G.: Zbl. Gyn. 1925:*49:*975.
4. FREUND, H.: Z. Geb. u. Gyn. 1912:*72:*97.
5. HEINRICIUS, G.: Zbl. Gyn. 1891:*15:*673.
6. KRAUS: Zbl. Bakt. usw. Ref. 1910:*48:*239.
7. KÜHNAU, W.: Berl. klin. Wschr. 1898:*28:*612.
8. KURTTIO, E.: Über Tetanus und sein Vorkommen in Finnland. Akademische Abhandlung. Helsinki. 1935.
9. LEHMAN, W., und FRAENKEL, E.: Arch. Gyn. 1923:*122:*692.
10. MARTIN, E.: Zbl. Gyn. 1906:*14:*395.
11. MEINERT: Arch. Gyn. 1893:*44:*381.
12. PROST: Zbl. Gyn. 1924:*20:*1087.
13. REINHARDT, A., und ASSIM, A.: Zbl. Bakt. usw. 1 Abt. Orig. 1909:*49:* 583.
14. ROTHSCHILD: Münch. med. Wschr. 1922:*69:*1011.
15. SATTLER, O.: Ein Fall von Tetanus nach kriminellem Abortus. Thesis. Tübingen. 1890.

16. Schneider, G. H.: Klin. Wschr. 1925:*4:*2438.
17. Schottmüller: Refer. nach Flechtner und Quast.
18. Seegert, P.: Zbl. Gyn. 1906:*30:*393.
19. Simon, W.: Zbl. Gyn. 1923:*47:*545.
20. Spiegel, R.: Arch. Gyn. 1914:*103:*367.
21. Steinitz, G.: Dtsch. med. Wschr. 1906:*32:*1419.
22. Mironescu and Bals: Refer. nach Lancet (London) 1938:*1:*1421.
23. TenBroeck, C., and Bauer, J.: J. exper. Med. 1922:*36:*261.
24. Rose: Refer. nach Kühnau.
25. Vaillard, L. et Rouget, J.: Ann. Inst. Pasteur, Par. 1892:*6:*385.
26. Waring: Refer. nach Kühnau.
27. Vinay: Lyon méd. 1891:*51.* Refer. nach Kühnau.
28. Völcker, E.: Zbl. Gyn. 1925:*49:*1625.
29. Zeissler, J.: Anaërobenzüchtung. Kolle, W., und v. Wassermann, A. Handbuch der pathogenen Mikroorganismen. Fischer und Urban & Schwarzenberg. Jena, Berlin und Wien. 3 Aufl. 1930:*10:*35.

SUBGROUPS A_1, A_2, AND A_1B, A_2B AND THEIR RELATION TO HEMAGGLUTININS PRESENT IN SEEDS OF CYTISUS SESSILIFOLIUS

By

ROLF KOULUMIES

(Received for publication July 11, 1949)

On the basis of observations made by K. O. Renkonen (2), according to which certain seed extracts contain anti-A_1 agglutinins and certain others anti-O (anti-A_2) agglutinins, Koulumies (1) has in a previous publication demonstrated that saline extract made of Vicia cracca seeds is as suitable as absorbed anti-A_1 sera for differentiation of subgroups A_1, A_2 and A_1B, A_2B.

The object of the present investigation is to study the suitability of saline extracts from seeds of Cytisus sessilifolius, for differentiation of the above mentioned subgroups. Renkonen (2) found Cytisus sessilifolius to contain anti-O agglutinins.

MATERIAL AND METHOD

The blood cells were obtained from the local Blood Service Center. The blood cells were taken in citrate blood and washed twice with saline. Only fresh blood cells were used.

The differentiation of subgroups was made by the method described in the previous paper (1).

The extract of Cytisus sessilifolius was prepared as follows:

The seeds were powdered by hand in a mortar. The extract was made of one part of seed powder to nine parts saline and kept

for two hours at 37° C. After centrifugation supernatant was used in agglutination tests with saline as a diluent.

The test was made by pipetting 0.1 ml of extract dilution and 0.1 ml of 2 per cent cell suspension into test tubes, which were kept for two hours at room temperature. The results were determined by the naked eye.

This reaction differs from those carried out with Vicia Cracca only in that the dilution began at 1: 10 and that the time of reaction was two hours, as against 1: 100 and one hour in the case of Vicia, depending on the relatively weaker reaction of the Cytisus. The results were controlled through titrating every time standard A_2 and 0 cells.

RESULTS

Subgroups. — A total of 100 specimens of blood group A cases were examined and found to belong to different subgroups as follows: 72 were certain A_1 cases in which the reaction to anti A_1 serum was clearly positive (+++ or ++) and to anti-O serum negative (—). 25 blood samples were certain A_2 cases in which the reaction to anti A_1 serum was fully negative (—) and which gave a clearly positive (+++ or ++) reaction to the anti-O serum. The remaining 3 specimens reacted in a different way, viz.:

In the case of A. H. the cells were not agglutinated with either anti-A_1, or anti-O serum and gave the absorbtion curve shown in Fig. 1 (indicating a close relation to the control curve of A_2).

Neither did case K. H. react to anti-A_1 and anti-O sera. The absorption curve appears in Fig. 2 (and corresponds to the control curve of A_2).

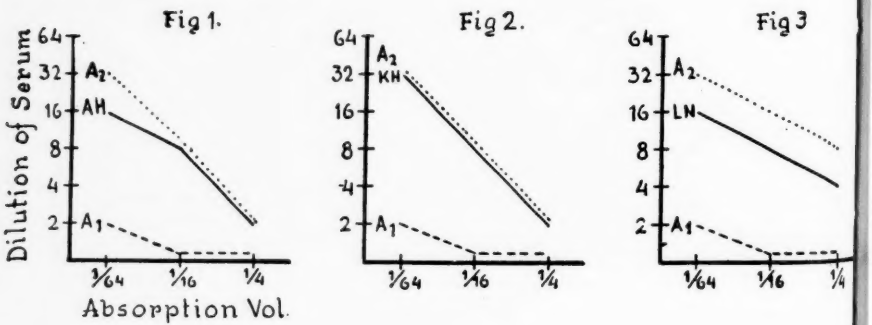

TABLE 1

TITRATION OF CYTISUS SESSILIFOLIUS EXTRACT

Extract Dilution	Number of Cases in Each Group					
	A_1	A_2	»Intermediates» A	A_1B	A_2B	0
> 1:10	72		2	35	15	
1:10						
1:20		1				
1:40		4	1			2
1:80		10				5
1:160		9				12
1:320		1				6
Totals	72	25	3	35	15	25

TABLE 2

TITRATIONS OF A CELLS WITH VICIA CRACCA EXTRACT

Dilution of Vicia extract	Number of Cases		
	A_1	A_2	»Intermediates» A
> 1:100		20	
1:100		3	2
1:200		1	
1:400		1	
1:800			1
1:1600	18		
1:3200	39		
1:6400	15		
Totals	72	25	3

Case L. N. gave a weak (±) reaction to anti-A_1 serum and a nearly as weak (+) reaction to anti-O serum. Its absorbtion curve is given Fig. 3 (and is between the A_1 and A_2 curves).

A total of 50 samples belonging to blood group AB were examined. 35 of these were of subgroup A_1 B and 15 of subgroup A_2B. No cases of »Intermediates» were found.

Titration with Seed Extract. — The agglutination of red cells caused by Cytisus sessilifolius extract proved much weaker than the corresponding reaction to the Vicia Cracca extract. While the Vicia titre rose to several thousands, the agglutination of Cytisus remained at the level of some hundreds. Furthermore, the Cytisus reaction was so much slower that it was necessary to keep the test tubes at room temperature for two hours in order to obtain a distinct reaction. As Cytisus titre we took the greatest dilution giving a positive reaction. Results are illustrated in Table 1.

A_2-cases reacted to the Cytisus extract like O-cells but a little weaker (Table 1). The Vicia titres of the same A_2 cases are illustrated in Table 2.

None of the A_1 cells examined reacted to the Cytisus extract. Their titre, evaluated on a basis of strong reaction on Vicia cracca, are illustrated in Table 2.

The AB group, too, was negative in its totality. Not even a weak reaction to Cytisus extract could be found in the 35 A_1B and the 15 A_2B cases examined.

Intermediates A-cases: Both A. H. and K. H. reacted with Vicia 1: 100 but *their reaction to Cytisus was fully negative.* In the third case, L. N., the Cytisus titre was 1: 40. The Vicia titre was 1: 800 in this case.

To *sum up* it seems that differentiation of subgroups A_1 and A_2 can be done with saline extract of Cytisus sessilifolius seeds as well as with anti-0 serum. In the three exceptional cases, in which conclusive results could not be obtained with anti-A_1 and anti-0 serum, the results with seed extract also remained inconclusive.

REFERENCES

1. KOULUMIES, R.: Ann. Med. Exp. Biol. Fenn. 1949:*27*:20.
2. RENKONEN, K. O.: Ann. Med. Exp. Biol. Fenn. 1948:*26*:66.

FROM THE WOMEN'S CLINIC AND THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

THE ALBUMIN AND GLOBULIN FRACTIONS OF THE SERUM OF NORMAL WOMEN, DETERMINED BY THE METHANOL PRECIPITATION METHOD

By

ANNA-LIISA ALHA

(Received for publication August 2, 1949)

In the investigation (1) in which I compared the albumin- and globulin fraction values of the serum protein obtained by the Na_2SO_4 precipitation method to those obtained by the methanol precipitation method, the results were concordant with the determinations carried out by Pillemer and Hutchinson (6). These show that the Na_2SO_4 precipitation method, which is in common use in clinical practice, gives considerably higher albumin values than the methanol precipitation method, which according to Pillemer and Hutchinson gives values equivalent to those obtained by the electrophoretic method. Thus the figures representing the albumin and globulin fractions of the serum protein obtained by the Na_2SO_4 precipitation method which are to be found in the literature do not seem to be the real values of the albumin and globulin fractions.

In this investigation I have carried out the determinations by the methanol precipitation method on the serum of forty normal women.

The material of this investigation consisted of the controls for my investigation of the proportion of the albumin and globulin fractions in pregnant women. This investigation will be published later. The subjects were clinically healthy nurses from

TABLE

	Tot. Prot. %	Alb. %	Glob. %	A/G	Non-prot. N of Ser.
1	7.310	4.360	2.950	1.470	30
2	7.600	4.152	3.448	1.175	30
3	8.212	4.818	3.394	1.410	30
4	7.460	3.772	3.688	1.025	30
5	7.145	3.590	3.555	1.012	30
6	7.005	3.662	3.342	1.096	30
7	8.002	4.642	3.360	1.381	30
8	8.387	4.355	4.032	1.080	30
9	8.265	4.660	3.605	1.292	30
10	7.090	4.065	3.035	1.339	30
11	7.642	4.597	3.045	1.509	26
12	7.397	3.617	3.780	0.956	26
13	7.417	4.250	3.167	1.342	34
14	7.015	3.777	3.238	1.166	34
15	7.460	4.057	3.402	1.192	30
16	7.617	3.670	3.947	0.929	30
17	7.005	4.142	2.862	1.447	30
18	7.495	4.520	2.975	1.522	30
19	7.512	4.034	3.478	1.159	30
20	8.002	4.509	3.493	1.290	30
21	6.760	4.226	2.534	1.668	30
22	6.970	4.192	2.778	1.509	30
23	7.460	4.205	3.255	1.292	30
24	8.108	5.220	2.888	1.807	30
25	7.915	4.170	3.745	1.114	30
26	7.635	4.463	3.172	1.408	30
27	8.073	3.488	4.385	0.760	30
28	7.233	4.234	2.999	1.412	30
29	7.418	4.409	3.009	1.465	34
30	7.565	4.398	3.168	1.388	30
31	7.355	4.363	2.993	1.458	30
32	6.935	4.188	2.747	1.525	30
33	6.568	3.260	3.208	1.016	30
34	7.600	4.328	3.272	1.322	30
35	7.443	3.838	3.605	1.064	30
36	7.785	4.674	3.111	1.506	34
37	7.093	4.066	3.028	1.344	30
38	8.060	4.503	3.557	1.266	30
39	7.688	3.742	3.947	0.949	30
40	7.698	4.393	3.305	1.333	34
Mean	7.332	4.190	3.318	1.285	30,3
Stand Dev.	0.459	0.395	0.606		

the Women's Clinic and members of the laboratory staff in the Department of Serology and Bacteriology, aged about 20—35 years. In one case the albumin — globulin proportion was 0.677, which was lower than in any other case. The nonprotein nitrogen was 60. Physical examination showed that this nurse suffered from nephropathy, so the case was excluded from this series.

Here are some values obtained by the neutral salt precipitation method which are commonly found in the literature:

	Alb. %	Glob. %	A/G
Rowe(8)	4.6—6.7	1.2—2.3	
Kolmer and Boerner (4) ..	4.0—5.0	2.0—2.5	1.5—2.5: 1
Helve etc. (3)	4.0—5.3	1.8—3.0	1.7: 1—3: 1

Women's albumin and globulin values in the literature are usually slightly differing from men's. Thus, for instance, according to Rinehart (1945) (neutral salt precipitation):

	Alb. %	Glob. %
Women	4.7—5.2	1.5—2.4
Men	4.0—5.8	1.6—3.8

The following mean values (neutral salt precipitation) obtained only on normal women may still be mentioned:

	Alb. %	Glob. %	A/G
Dienst (2)	3.95	2.89	
Møller-Christensen etc. (5)	4,67	2.49	1.94

My investigation gave the following results:

	Alb. %	Glob. %	A/G
	3.260—4.818	2.534—4.385	0.760—1.807
Mean value	4.190	3.318	1.285

The proportion of the albumin and globulin fractions obtained by the methanol precipitation, when compared to the proportions given above (neutral salt precipitation), is found to be much lower.

SUMMARY

The proportion of the albumin and globulin fractions has been determined by the methanol precipitation method on forty normal women aged 20—35. The result of this investigation is that the proportion is considerably lower than in those reported cases which have been examined by neutral salt precipitation.

REFERENCES

1. Alha, A.-L.: Ann. Chir. Gyn. Fenn. 1949. *38*. Suppl. No. 3. p. 6.
2. Dienst, A.: Arch. f. Gynäk. 1918:*109:*669.
3. Helve, O., Koulumies, R. etc.: Introduction to Clinical Laboratory Technique (Finnish). Helsinki 1947.
4. Kolmer, J. A. and Boerner, F.: Approved Laboratory Technic. New York 1945.
5. Møller-Christensen, E. and Thygesen, J. E.: J. Obstet. Gynac. Brit. Emp. 1946:*53:*328.
6. Pillemer, L. and Hutchinson, M. C.: J. biol. Chem. 1945:*158:*299.
7. Rinehart, R. E.: Amer. J. Obstetr. 1945:*50:*48.
8. Rowe, A. H.: Arch. int. Med. 1916:*18:*455.

FROM THE WOMEN'S CLINIC AND THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

DETERMINATION OF THE ALBUMIN AND GLOBULIN CONTENTS OF HUMAN SERUM BY PILLEMER AND HUTCHINSON'S METHANOL PRECIPITATION

A METHODICAL STUDY

By

ANNA-LIISA ALHA

(Received for publication August 9, 1949)

I have carried out investigations on the albumin and globulin fractions of the blood serum in women, using the methanol precipitation of Pillemer and Hutchinson (3).

In this work I examined the accuracy of the method.

I used three women as subjects.

I carried out 10 parallel determinations on each subject in the way described in my earlier article (1, 2). As the object of the investigation was to examine the differences caused by the technique in the routine determination, these determinations were carried out in the connection of other investigations.

In each case the mean value and the standard error of the mean value was determined by means of the probability calculus presented on the following page.

The following tables give a summary of all three cases.

The results show that the standard errors of the mean values do not differ greatly from one another.

In the tables showing the parallel determinations some results have been underlined. There are two underlined determinations in cases 1 and 2, and 3 in case 3.

These cases apparently show a greater technical error than usual, for they do not fall inside the values which according to

CASE 1.

Tot. Prot.

x	$v=(x-7.250)$	v^2
7.425	+0.175	0.030625
0.268	+0.018	0.000324
0.250	—	—
0.163	−0.087	0.007569
0.215	−0.035	0.001225
0.250	—	—
0.110	−0.140	0.019600
0.163	−0.087	0.007569
0.355	+0.105	0.011025
0.180	−0.070	0.004900
	−0.121	0.082837

$\Sigma v = -0.121$ $\Sigma v^2 = 0.082837$

$\frac{(\Sigma v)^2}{n} = 0.0014641$

$\therefore \Sigma (x-\bar{x})^2 = 0.081373$

$s_1^2 = 0.009041$

$s_1 = 0.095$ (stand. dev.)

$\frac{\Sigma v}{n} = -0.0121$

$\therefore \bar{x} = 7.2379$ (mean value)

standard error $\frac{s_1}{\sqrt{n}} = 0.030$ %

Alb.

y	$u=y-4.625$	u^2
4.678	0.053	0.002809
0.653	0.028	0.000784
0.623	−0.002	0.000004
0.450	−0.175	0.030625
0.653	0.028	0.000784
0.625	—	—
0.660	0.035	0.001225
0.538	−0.087	0.007569
0.678	0.053	0.002809
0.625	—	—
		0.046609

$\Sigma u = -0.067$ $\Sigma u^2 = 0.046609$

$\frac{(\Sigma u)^2}{n} = 0.0004489$

$\Sigma (y-\bar{y})^2 = 0.046160$

$s_2^2 = 0.005129$

$s_2 = 0.072$ (stand. dev.)

$\frac{\Sigma u}{n} = -0.0067$

$\therefore \bar{y} = 4.6183$ (mean value)

standard error $\frac{s_2}{\sqrt{n}} = 0.023$

Glob. mean value $= 7.2379 - 4.6183 = 2.6196$

standard error $= \sqrt{\frac{s_1^2}{n} + \frac{s_2^2}{n}} = \sqrt{0.0014170} = 0.0376$

	Tot. Prot.	Alb.	Glob.
5 % probability that the real mean value is outside the limit $\bar{x} + 2.26 \cdot \frac{s}{\sqrt{n}}$	7.170 — 7.306	4.566 — 4.670	2.535 — 2.705
1 % » » » » » » » » » » » $\bar{x} + 3.25 \cdot \frac{s}{\sqrt{n}}$	7.140 — 7.335	4.544 — 4.692	2.497 — 2.742
0.1 % » » » » » » » » » » » $x + 4.78 \cdot \frac{s}{\sqrt{n}}$	7.095 — 7.381	4.506 — 4.728	2.440 — 2.799

TABLES

CASE 1. (MAY 1949) NON-PROT. N OF SER. 30 MG PER CENT.

No. of Parall. Det.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Tot. prot. %	7.425	7.268	7.250	7.163	7.215	7.250	7.110	7.163	7.355	7.180
Alb. %	4.678	4.653	4.623	4.450	4.653	4.625	4.660	4.538	4.678	4.625
Glob. %	2.747	2.615	2.627	2.713	2.562	2.625	2.450	2.625	2.677	2.565
Alb./Glob.	1.703	1.778	1.759	1.640	1.815	1.762	1.900	1.729	1.747	1.803

CASE 2. (JUNI 1949) NON-PROT. N OF SER. 30 MG PER CENT.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Tot. prot. %	7.985	7.950	8.020	7.963	8.156	8.050	8.106	7.963	7.985	8.125
Alb. %	4.643	4.450	4.643	4.678	4.643	4.695	4.660	4.550	4.713	4.695
Glob. %	3.342	3.500	3.377	3.285	3.513	3.355	3.446	3.413	3.273	3.430
Alb./Glob.	1.389	1.271	1.375	1.424	1.350	1.399	1.352	1.333	1.439	1.369

CASE 3. (JULI 1949) NON-PROT. N OF SER. 30 MG PER CENT.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Tot. prot. %	5.863	5.763	5.675	5.881	5.790	5.706	5.688	5.638	5.675	5.790
Alb. %	3.519	3.313	3.413	3.363	3.344	3.275	3.225	3.256	3.225	3.313
Glob. %	2.344	2.450	2.262	2.518	2.446	2.431	2.463	2.382	2.450	2.477
Alb./Glob.	1.459	1.352	1.509	1.336	1.367	1.347	1.309	1.367	1.316	1.333

Cases	Tot. prot. %		Alb. %		Glob. %		Alb./Glob
	mean value	standard error	mean value	standard error	mean value	standard error	mean value
1.	7.238	0.030	4.618	0.023	2.620	0.038	1.764
2.	8.030	0.023	4.637	0.024	3.393	0.038	1.370
3.	5.747	0.026	3.325	0.029	2.422	0.040	1.370

the probability calculus would be limit values. If we suppose that all values fall inside the limits mean value $\pm 3 \times$ the standard error of the mean value, which limits in case 1 for the total protein were (7.238 $\pm 3 \times 0.03$ %) 7.148—7.328, the value 7.425 of the total protein obtained in case 1 is beyond these limits.

REFERENCES

1. Alha, A.-L.: Ann. Chir. Gyn. Fenn. 1949. *38*. Suppl. No. 3. p. 6.
2. Alha, A.-L.: Ann. Med. Exp. Biol. Fenn. 1949.*27*:189.
3. Pillemer, L. and Hutchinson, M. C.: J. Biol. Chem. 1945:*158*:299.

FROM THE PHYSIOLOGICAL INSTITUTE AND CHILDREN'S CLINIC,
UNIVERSITY OF HELSINKI

THE FRUCTOSE OF SHEEP FOETAL BLOOD

By

M. J. KARVONEN

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Fructose is normally present in the animal body as a component of several compounds. Free fructose, however, does not occur in significant amounts in the blood of adult mammals. When administered to them, fructose rapidly disappears from the circulation; only if the liver has been damaged, the disappearance of fructose is delayed.

During the foetal life of some mammalian species fructose, however, is a normal component of the blood sugar. Bacon & Bell (3) were able to isolate and to identify fructose in the foetal blood of sheep. Partridge (16) showed with chromatographic methods that fructose, glucose and inositol are the only sugars of foetal sheep blood; of these inositol is non-reducing, and not included in the usual sugar determinations. Cole & Hitchcock (7) and Hitchcock (10) have determined the glucose and fructose concentration in the blood of sheep foetuses at various ages. Whereas the maternal circulation of the pregnant ewe contained practically no fructose, a great proportion of the foetal blood sugar — often more than half — was fructose. The level of fructose in the foetal blood was found to fall towards term and that of glucose to rise from a low level to one normal for the maternal blood. It was also demonstrated that the maternal blood traversing the placenta losed glucose and that the foetal blood correspondingly gained approximately an equal amount of it. The figures for the fructose level in the umbilical vessels were not consistent, and one could not judge whether there was any fructose gradient from the placenta towards the foetus or

in the opposite direction. Within the first hours after delivery, the fructose disappeared from the blood.

The results of these previous investigations were a stimulus for further studies. The following problems were considered: *Is the fructose a substance actively utilised by the foetal organism* or is the foetus incapable to deal with this physiological sugar which in adult organism rarely accumulates to measurable concentrations? No information on this point was found in the literature. In the present work, this question was approached experimentally by using fructose and glucose *tolerance tests*. Secondly, *where from the fructose comes to the foetal blood?* In order to study this question, Hitchcock (11) has carried out a number of incubation experiments with pieces of placenta and foetal liver and lung. In these experiments, occasionally the fructose concentration of foetal lung increased slightly on incubation, but this finding was not constant. In the present work an attempt was made to investigate the rôle of *lung and placenta in the fructose metabolism* more or less *in situ*.

MATERIAL AND METHODS

Sheep of the standard Finnish breed were used as experimental animals. This breed differs from the Welsh sheep — the breed mainly used by the Cambridge school of the late Sir Joseph Barcroft — by having generally twin instead of single pregnancies. The exact dates of the tupping were not known. The approximate foetal ages were estimated by using the age-length graph given by Barcroft (4) for his series.

The ewes were anaesthetized with 'Nembutal' or with a 10 or 20 per cent solution of Na-diaethylbarbiturate. The latter anaesthetic was used in lack of 'Nembutal', on the suggestion of Vartiainen (22), and it proved a fairly satisfactory anaesthetic for the tolerance experiments, as its action lasted longer than that of 'Nembutal'. The dose was adjusted by administering slowly the anaesthetic intravenously. In order to lengthen the action of 'Nembutal' a depot of a few cc. of it was often injected subcutaneously or intramuscularly, and again repeatedly during long experiments. Both anaesthetics increased the blood sugar of the mother, but most often only moderately, as shown in the examples given in Table I.

TABLE I

MATERNAL VENOUS BLOOD SUGAR DURING ANAESTHESIA

Number	Time	Anaesthetic	Total Blood Sugar Mg Per Cent
H 4	10.10		48
	10.12	Nembutal	
	10.55		49
	12.05		59
H 6	10.25		46
	10.30	Na-diaethylbarbiturate	
	11.15		81
	11.42		71
H 9	12.16		60
	12.20	Na-diaethylbarbiturate	
	12.45		67
	13.14		71
	14.00		76
H 11	10.07		61
	10.10	Na-diaethylbarbiturate	
	10.54		67
H 26	11.30		43
	11.35	Nembutal	
	12.10		67
	12.50		80
H 27	11.15		41
	11.18	Nembutal	
	11.23		39
	12.15		41

A caesarean section was performed on the sheep in a thermostatic 0.9 per cent NaCl bath of a special design, at +38° C. (The bath was made by Messrs. Merivaara Oy, Helsinki, and the apparatus for the thermostatic control was installed by Messrs. Santasalo-Sohlberg Oy Ab, Helsinki.) The foetuses were delivered into the bath, where they remained in life and in connection with the placental circulation throughout the experiment. Blood samples were taken from the umbilical veins. In old foetuses, these contracted very easily. In order to avoid this, samples were some-

times taken through a cardiac punction. Treating the umbilical cord with 40 per cent formaldehyde (12) was also found effective for avoiding the contraction of the vessels on puncture.

For sugar determinations, the deproteinisation of the blood was performed with the cadmium precipitation (9). The total sugar was determined in the protein-free filtrate according to Somogyi (21). From the sheep No. H 21 onwards this method was used in combination with Nelson's (15) colour reagent, making the readings with a Coleman electrical spectrophotometer, at 5,200 Å. Some batches of the Somogyi reagent gave higher reduction values with fructose than with glucose; therefore, both glucose and fructose were always used as standards. The special advantage of using Somogyi's method on blood is, that it has been shown to indicate the fermentable substance only. This was ascertained also on some samples of foetal sheep blood. (Method: see (13). The results of the fermentation experiments are shown in Table II. When any residual reduction remained after the fermentation, it was evidently due to the yeast, as the reduction was present also in the controls.

TABLE II

THE RESULTS OF FERMENTATION EXPERIMENTS ON SHEEP FOETAL BLOOD

Number of Sheep		Total Reducing Substance as Mg Per cent Hexose	Fructose Mg Per Cent
H 8	Blood	201	170
	Blood + yeast	19	0
	Distilled water + yeast	21	0
	100 mg per cent fructose + yeast	18	0
H 7 a	Blood	190	148
	Blood + yeast	0	0
b	Blood	165	116
	Blood + yeast	0	0
H 6 a	Blood	110	80
	Blood + yeast	3.5	0
b	Blood	245	150
	Blood + yeast	5.5	0
	100 mg per cent fructose + yeast	3.2	0

The fructose concentration was estimated with Cole's (6) modification of Roe's (19) colorimetric method. The essential features of this method have been published by Bacon and Bell (2) and by Karvonen and Somersalo (13). From the sheep No. H 21 onwards the readings were made with a Coleman spectrophotometer, at 4,700 Å, instead of the stufenphotometer previously used. The sugar analyses were performed on a 'macro' scale, using 1 cc. blood for the complete determination. These were made in duplicate. The difference between the total sugar and fructose concentrations is expressed as glucose. This is justified because there remained after the yeast fermentation experiments no more residual reduction in the blood than in the controls.

RESULTS

Normal Fructose Levels. — The values for the fructose and glucose levels in foetal blood are presented in Table III. These values conform in general with those reported by Hitchcock (10).

Fructose and Glucose Tolerance Tests. — For finding out, whether the foetus is capable of disposing of fructose or glucose att all, the disappearance of intravenously injected fructose and glucose from the foetal blood was followed. In order to compare the rate of disappearance of the sugars from the foetal circulation with the corresponding rate during the post-natal life, tolerance tests were performed also on adult sheep and on young lambs.

(*a*) *Adult sheep.* Altogether three fructose and three glucose tolerance tests were made on five ewes. Their results are shown in fig. 1 and 2. The dose used was 1.0 g per kg body weight. The sugars were injected into the jugular vein, as a 20 per cent solution. If the rate of the injection was too rapid, the sheep exhibited central nervous system symptoms, got excited and made incoordinated movements of the neck. The symptoms passed in a few minutes.

In the *glucose experiments*, one hour after the injection the blood *glucose level* was on an average 181 mg per cent (176, 184, 184) *above* the starting values. In the fructose tolerance tests, after one hour the *fructose level* was correspondingly 69 mg per cent (79, 68, 61) and the *glucose level* 68 mg per cent (114, 62, 28) *above* the pre-experimental level.

(*b*) *Foetuses.* Six foetuses were used for fructose and three for

TABLE III

THE ESTIMATED AGES OF THE FOETUSES. THE BASAL FRUCTOSE AND GLUCOSE VALUES

No. of Ewes	Number of Foetuses	Estimated Age Days	Fructose Mg Per Cent	Glucose Mg Per Cent
H 22	3	49	123	2
H 23 a	2	69	148	20
b			143	7
H 9	1	105	72	38
H 5 a	2	105	68	53
b			72	48
H 4 a	3	106	67	37
b			52	30
c			54	38
H 10 a	2	110	30	39
			21	48
H 25 a	2	110	60	34
b			93	31
H 7 a	2	113	80	52
b			84	38
H 6 a	2	121	65	59
b			59	92
H 8	1	123	140	62
H 11	1	125	62	40

glucose tolerance experiments. The results of these experiments are shown in fig. 3 and 4. In two cases (sheep No. H 7 and H 25) a fructose and a glucose experiment was performed simultaneously on twins. In one of the experiments (sheep No. H 6) one of twins was given a fructose tolerance test, and the blood sugars of its twin were followed as a control; they kept fairly constant, and there was only a slow rise of glucose from 59 to 77 mg per cent during 1¼ h. The dose used was estimated to equal 1 g of sugar per kg foetal body weight.

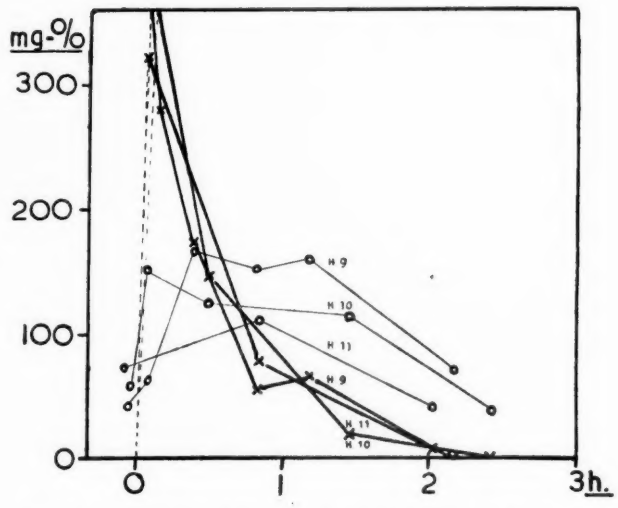

Fig. 1. — Fructose tolerance tests on three adult sheep.
Blood fructose: x———x
Blood glucose: o———o

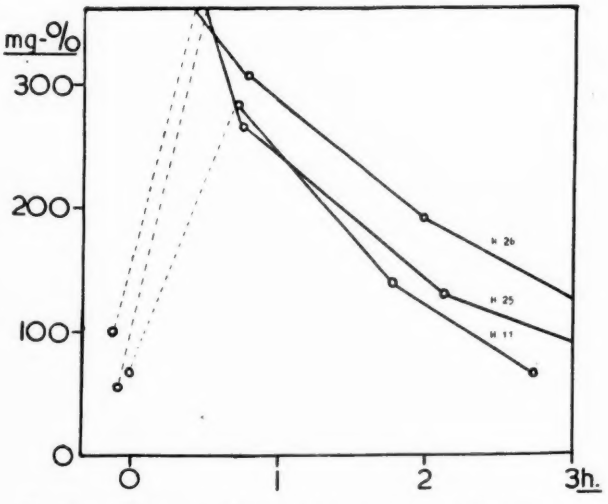

Fig. 2. — Glucose tolerance tests on three adult sheep.
Blood glucose: o———o

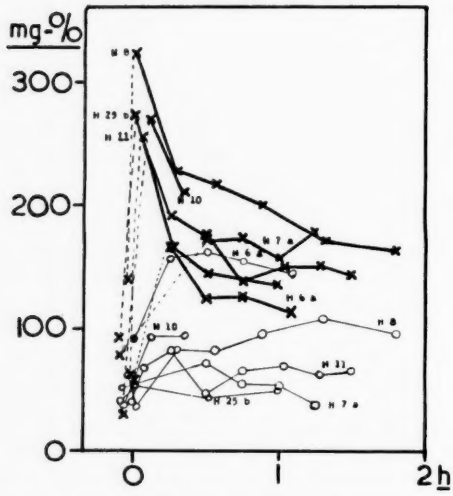

Fig. 3. — Fructose tolerance tests on six sheep foetuses. The foetal ages: H 10—110 days, H 25 b—110 d., H 7a—113 d., H 6 a—121 d., H 8—123 d., H 11—125 d.
Blood fructose: x———x
Blood glucose: o———o

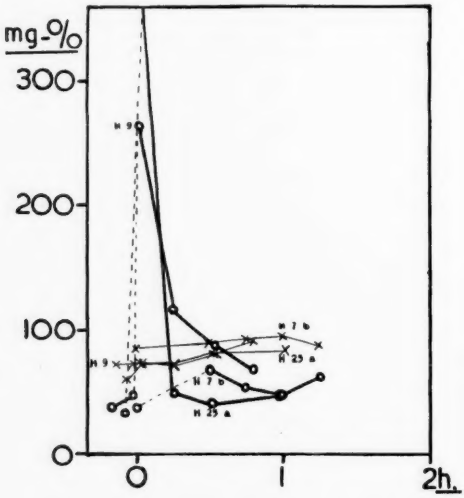

Fig. 4. — Glucose tolerance tests on three sheep foetuses. The foetal ages: H 9—105 days, H 25 a—110 d., H 7 b—113 d.
Blood fructose: x———x
Blood glucose: o———o

One hour after the injection, the fructose tests showed the following situation: the *fructose level* was on an average 63 mg per cent (77, 52, 57, 86, 47) above the starting level, and the *glucose* had risen on an average by 28 mg per cent (1, 29, 37, 54, 18). In the *glucose experiments* the corresponding figures are: glucose 12 mg per cent (9, 14, and 13: by extrapolation) above the pre-experi-

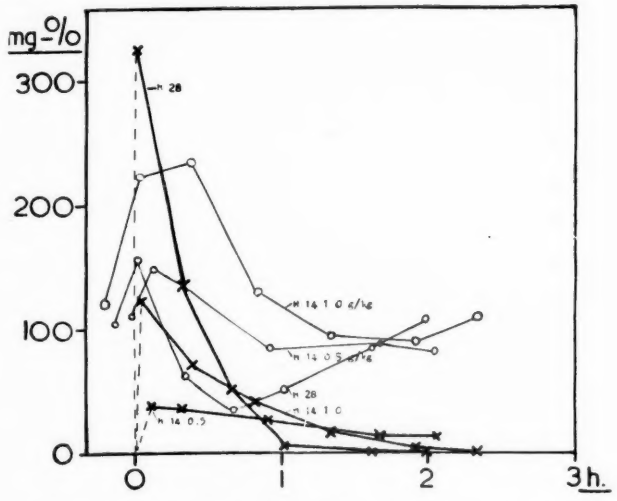

Fig. 5. — Fructose tolerance tests on two young lambs.
H 14 0.5 g/kg body weight administered at the age of 6 days.
H 14 1.0 g/kg body weight administered at the age of 13 days.
H 28 1.0 g/kg body weight administered at the age of 35 days.
Blood fructose: x———x
Blood glucose: o———o

mental level, and an average rise of *fructose* by 17 mg per cent (11, 24, and 17: by extrapolation).

A comparison of the maternal and foetal *fructose tolerance tests* shows, that *the extra fructose disappeared with the same rate from both the adult and foetal circulation, whereas the consequent rise of glucose was less in the foetuses. In the glucose tests, the glucose disappeared very rapidly indeed from the foetal circulation;* in the foetus the fructose level rose slightly, but no fructose appeared in the adult blood.

(*c*) *Young lambs.* Three fructose tolerance tests were performed on two young lambs. Their results are shown in fig. 5. The fall of the fructose level seems to be more rapid than in adult animals:

after one hour the *fructose level* was 26, 34 and 6 mg per cent, and the *glucose level* was already 24, 2 and 52 mg per cent *below* the starting level. No further experiments were performed on lambs, as it was felt that they would hardly serve to elucidate the present problems.

Lung. — The fairly rapid disappearance of injected extra fructose from the foetal circulation made it probable that the normal fructose of the foetal blood is produced — in some part of the foetal organism — at a rate considerably in excess of the corresponding production during the post-natal life. The rapid loss of fructose after delivery indicates that there occurs at birth a radical change in the conditions of the fructose production. In a search for a site responsible for the fructose production several possibilities come into question. First, the whole foetus experiences a radical improvement of its oxygen supply. In the lungs the rise of the oxygen pressure is probably more marked than in any other organ. Second, the foetus loses a big and important organ, the placenta. And third, with the occlusion of the *ductus venosus* the circulatory conditions of the liver change also considerably. These alterations perhaps are the most obvious ones to be investigated when attempting to find the site of the fructose formation.

In the present study, the rôle of the lungs was investigated by excluding them from the circulation. This was performed in an experiment on triplets (No. H 4), 106 days old. The chest of two of the foetuses was opened on both sides and the sternum with parts of the ribs was removed. The edges of the opening were compressed with big clamps, in order to prevent bleeding. Thereafter a strong ligature was brought round the hilus of each lung and the lungs were tied up firmly. In order to control that the closure of the pulmonary circulation had been effective, the lungs were cut away. In the first foetus, the operation led to an uncontrolled bleeding and the animal soon died. However, the state of the second foetus remained satisfactory for about an hour after the operation. The third foetus was kept as a non-operated control.

The results of the sugar analyses are given in Table IV. No significant change in the blood sugar levels occurred. The results of this experiment, therefore, indicate that the foetal lung is not essential either for the production or for the utilisation of blood fructose.

TABLE IV

RESULTS OF AN EXPERIMENT IN WHICH THE LUNGS OF A FOETUS (H 4 B) WERE EXCLUDED FROM THE CIRCULATION

Time		Total Blood Sugar Mg Per Cent	Fructose Mg Per Cent	Glucose Mg Per Cent
	Foetus b, operated upon			
11.01		82	52	30
11.18	The lungs are ligated			
11.30		96	61	35
11.54		115	62	53
12.08		103	55	48
	Foetus c, control			
11.02		92	54	38
11.18		103	58	45
11.30		103	53	50
11.47		99	53	46

Placenta. — After the ligation of the cord, the foetal blood in the placental vascular bed does not clot for a considerable time. It remains there under the tonic pressure of the contracting umbilical and placental vessels. Samples of it can be taken easily after the ligation.

Under these conditions, the composition of the placental blood did not remain constant. When successive samples were taken, a notable decrease in the haemoglobin concentration took place. At the same time the samples had the bright colour of oxygenated blood. This suggests that both fluid and oxygen penetrated from the maternal side to the separated foetal vascular bed.

The sugars of such samples were analysed, and the results of the analyses are compiled in Table V. They show that the concentration of fructose did not fall in proportion to the drop in haemoglobin, or that it sometimes even rose in the separated vascular bed.

In Hitchcock's (10) series, there was an equivocal glucose gradient both in the uterine and in the umbilical vessels indicating a substantial transfer of glucose to the foetus. At the same time, however, the corresponding figures for fructose varied irregularly, and the average gradient in the umbilical vessels was practically zero. In the uterine vessels the fructose gradients were very small if any at all; the direction of the gradient was in three

TABLE V

BLOOD FRUCTOSE, GLUCOSE, AND HAEMOGLOBIN CONCENTRATION IN THE SEPARATED PLACENTAL VASCULAR BED

No. of Sheep	Time		Fructose Mg Per Cent	Glucose Mg Per Cent	Hb G/100 ml
H 8	12.49	On tying the cord	145	105	5.25
	13.08		143	57	6.6
	13.30		163	60	1.2
H 9	14.30	On tying the cord	97	39	4.5
	14.55		97	50	1.35
H 23	11.00	On tying the cord	139	69	3.75
			120	73	1.2

cases towards the mother, in one towards the foetus, and in two the level in the vein and the artery was the same.

Hitchcock's findings were confirmed in the present work. The different arterio-venous gradients are shown in Table VI. In

TABLE VI

MATERNAL UTERINE AND FOETAL UMBILICAL ARTERIO-VENOUS GRADIENTS FOR FRUCTOSE AND GLUCOSE

Sheep No.	H 22	H 21	H 23	H 26 a H 26 b	H 27	H 25 a H 25 b
Foetal Age Days	49	52	62	95	98	110
Fructose: Uterine Artery-Uter. Vein, while the Foetus is still Attached	−0.8	+1.0	+1.2	±0	−0.6	±0
Fructose: Uterine Artery-Uter. Vein, after the Foetus has been Removed	—	—	+1.1	−2.6	−0.2	−0.8
Glucose: Uterine Artery-Uter. Vein, while the Foetus is still Attached	+11	+1.8	−0.6	+11	−4	+20
Glucose: Uterine Artery-Uter. Vein, while the Foetus has been Removed	—	—	+12	+5	+11	+2
Fructose: Umbilical Artery-Umbil. Vein	—	—	—	−10 −2	− 3	+15 + 9
Glucose: Umbilical Artery-Umbil. Vein.....	—	—	—	− 1 −7	+2	−12 −14

combination with Hitchcock's figures, they indicate quite clearly the different behaviour of glucose and fructose.

The foetal fructose and glucose tolerance curves were strikingly different. In addition, the demonstration of an equivocal transfer of fructose from the foetus to its mother has failed, whereas there is no doubt about the leakage of glucose in the opposite direction. These findings were a stimulus for an attempt at a direct comparison of the permeability of placenta *in situ* to these two sugars. Two experiments were performed. In one of them, fructose (1.25 g/kg foetal body weight) was injected into the circulation of a foetus (H 29, foetal age 115 days), and samples were taken from the small maternal veins coming directly from a cotyledon. Arterial samples were also taken from the mother, in order to determine the concentration of sugars in the blood coming to the cotyledon. The time interval between these two samples was reduced to a minimum. In an analogous experiment glucose (1.3 g/kg foetal body weight) was used; the foetus (H 30) was 125 days old.

In the fructose experiment the foetal fructose level varied between 335 and 290 mg per cent, when the cotyledon samples were drawn. In the maternal arterial blood the fructose level was, of course, near to zero. (There is in the maternal blood some chromogene, equal to about 5 mg per cent fructose, when the present photometric technique is used; this colour reaction, however, is only slightly weakened by fermentation, and most of it is therefore due to substances other than fructose and glucose. In calculating arterio-venous gradients it has been assumed that the unspecific components do not affect the true fructose differences in any way.) In spite of the great difference between the foetal and maternal fructose levels there was only a slight leakage of fructose into the vein of the cotyledon. Two samples were taken: in one of them the a-v. gradient was $\pm$ 0, and in the other -4.6 mg per cent.

In the corresponding glucose experiment one sample was drawn 9 min. after the injection of the glucose solution. In this sample the glucose level in the blood coming from the cotyledon was 11 mg per cent *above* that in the general circulation. Another sample was taken 27 min. after the injection; the glucose level in the vein of the cotyledon was already 8 mg per cent *below* the arterial level.

DISCUSSION

The sugar *tolerance curves of the adult sheep* resemble essentially those obtained on man or on the common experimental animals. In the fructose tests, the blood fructose level rapidly fell down to zero, while the blood glucose showed a considerable, if fairly variable, rise. In the glucose tests the decrease of the total blood sugar was slower than in the fructose experiments, and no fructose appeared in the circulation of the sheep.

The sheep *foetus* shows a *fructose tolerance curve*, which has a similar form as that of the adult. The only obvious difference is that the foetal fructose curves lie higher up; the basal level is not zero, but a much higher one. The consequent rise of the glucose level was, however, less than in the fructose experiments on adult sheep.

In the *glucose tolerance tests*, the glucose disappeared from the foetal circulation much more rapidly than from that of the adult. There occurred a slight rise of the blood fructose, but similar rises were seen on mere exposure of the foetus. (A slow rise of fructose on exposure might also have been due to a rise of the maternal blood glucose during the operation?)

In the interpretation of the foetal tolerance curves two factors must be taken into consideration: the utilisation of the sugar by the foetus and its leakage through the placenta to the maternal circulation. There are plenty of data for the *permeability of the placenta* of sheep *for glucose*, in Hitchcock's (10), Passmore and Schlossman's (17) and in the present data. The permeability demonstrated has been towards the foetus, but there are in the placenta no histological structures that one is used to connect with uni-directional permeability. In rabbit and cat the permeability to glucose both ways has actually been established (20). Therefore, the most obvious explanation for the rapid fall of the glucose tolerance curve would be a leakage of glucose through the placenta to the maternal side. This assumption also explains, why the rise of glucose in the foetal fructose tests remains less than in the adult sheep; the glucose crosses the placenta, and therefore the rise in the glucose level is relatively small.

No equivocal *transfer of fructose through the placenta* has been demonstrated in normal conditions. This is the more remarkable, as fructose values are based on direct determinations, and those of

glucose to a difference between two figures. The actually observed permeability for fructose was also quite small, although the fructose level on the foetal side was raised until 300 mg per cent. One cannot avoid the tentative conclusion that the permeability of placenta for fructose is less than for glucose. This conclusion implies further, that the different permeability for fructose and glucose can not be due to physical factors — the molecules are of the same size — but must be accounted for by *an active transfer* of the sugars, at least *of glucose*. This view agrees well with the conditions of the absorption of the different hexoses through the intestinal mucosa: glucose is more rapidly absorbed than fructose, thanks to the rapid rate of phosphorylation of glucose (8, 23). It is also known that placenta is well equipped with phosphatase.

The present interpretation is diametrically opposed to the theory of Schlossman (19) and of several other workers (1, 5), who regard the passage of glucose through placenta as a purely physical process. The presented theory requires, of course, still to be substantiated in experiments designed directly for that purpose.

According to this new theory, the *disappearance of injected fructose* from the foetal circulation proceeds essentially through a process analogous to the utilisation of fructose by the adult animal. The 'spontaneous' occurrence of fructose in the foetal blood suggests that fructose is formed somewhere in the foetal organism at a rate considerably above the corresponding rate during post-natal life. Therefore, in a fructose tolerance test the foetus may possibly be dealing with more fructose than an adult, given the same dose per kg body weight. Actually, the few experiments on young lambs show a definitely faster disappearance of fructose than is observed in the adult sheep. This is in a good agreement with the above suggestion: a young lamb would have its high fructose tolerance as a legacy from the foetal period.

When fructose is injected as a hypertonic solution into the foetal circulation, it might be expected that a *transfer of water* from mother to foetus across the placenta would tend *to equalise the osmotic pressures* on the both sides. (There is all the reason to believe that water can pass through the placenta more easily than a molecule as big as fructose.) This transfer of water would lead to an immediate drop in the glucose level, followed later on by the

usual rise. In only one of the experiments an immediate drop of the glucose level was observed; its occurrence in other experiments can, of course, not be definitely excluded.

In the blood of the foetal sheep, the *presence of fructose* must be ascribed to some special factor or condition connected with the foetal life. At the end of the first half of the gestation, fructose is by far the main component of the blood sugar, whereas later on its level has a tendency to fall. If *morphological correlations* for this tendency are sought for, the analogous development of the *placenta* as compared to the foetus must be considered: the placenta grows and develops mainly during the first half of the pregnancy, whereas the growth of the foetus itself occurs substantially during the second half of the gestation.

At birth, the foetus soon loses the fructose from its blood. Again, the loss of fructose and of placenta occur simultaneously. This may be regarded as another piece of indirect evidence for the hypothesis that placenta is the site of the foetal fructose formation.

The *direct evidence* given in the present investigation for this hypothesis is meagre and must be taken with some reservation. The finding that fluid containing fructose appears in the separated placental vascular bed, may not necessarily indicate that the fructose is formed in the placenta. First, there might occur a spontaneous separation of cells and plasma in the placental vessels so that the plasma would be more readily sampled, thus giving rise to relatively high fructose values. This explanation does not seem very likely. Second, the fructose might diffuse from the placental tissue without, however, being formed there. This objection can not be entirely ruled out. It would imply, that fructose is not utilised by the placental tissue, but only passively stored there, diffusing back into the vessels when the circulation had stopped.

Another piece of similar direct evidence would be the demonstration of a leakage of fructose to the maternal circulation, after the removal of the foetus. Three of the figures of Table VI indicate such a leakage, whereas in one experiment the gradient was of the opposite sign. The latter of the above objections concerns also this method; the fructose may not be produced in the placenta, even if it diffuses to the blood from it. In addition, the arterio-venous gradient method has its inherent limitations. Both on foetal and maternal side the venous samples may not be representative of the

total venous blood coming from placenta. A time factor may also lead to experimental errors, as the arterial and venous samples can not easily be taken exactly at the same time. Still, considering all these objections, the available evidence seems to suggest that some fructose may be formed in the placenta. Whether placenta is the main or sole site of the foetal fructose formation, can not be decided on the basis of the present results.

SUMMARY

1. Intravenous glucose and fructose tolerance tests were performed on adult and foetal sheep and on young lambs.

2. In the glucose tolerance tests, the glucose level of the foetus decreased very much faster than that of the adult sheep. A slight rise of blood fructose occurred in the foetus.

3. In the fructose tolerance tests the return of the fructose to the pre-experimental level occurred with the same speed in the adult and the foetus. The consequent rise of blood glucose was less in the foetus.

4. In fructose tolerance tests on young lambs the fructose level fell faster than in adult and foetal sheep. The consequent rise of glucose passed rapidly.

5. The exclusion of lungs from the foetal circulation did not affect the levels of fructose and glucose in blood.

6. When repeated samples were taken from the separated placental vascular bed, the level of fructose did not fall in proportion to a fall in the haemoglobin concentration.

7. When samples were taken from the maternal veins of a cotyledon after an injection of fructose or glucose into the foetal circulation, the results suggested a greater placental permeability for glucose than fructose.

8. All the results agree best with the theory that placenta is more permeable for glucose than fructose.

9. The possible formation of fructose in the placenta is discussed.

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REFERENCES

1. Anselmino, K. J.: Arch. Gynäk. 1929:*138:*710.
2. Bacon, J. S. D., and D. J. Bell: Biochem. J. 1946:*40:*XLII.
3. Bacon, J. S. D., and D. J. Bell: Biochem. J. 1948:*42:*397.
4. Barcroft, J.: Researches on Pre-natal Life. 1946. Oxford: Blackwell Scientific Publications.
5. Brandstrup, E.: Acta Obst. Gynec. Scand. 1929:*8:*490.
6. Cole, S. W.: See (2) and (14).
7. Cole, S. W., and M. W. S. Hitchcock: Biochem. J. 1946:*40:*51.
8. Cori, C. F.: J. biol. Chem. 1925:*66:*691.
9. Fujita, A., and D. Iwatake: Biochem. Z. 1931:*242:*43.
10. Hitchcock, M. W. S.: J. Physiol. 1949:*108:*117.
11. Hitchcock, M. W. S.: Personal communication.
12. Huggett, A. ST. G.: Personal communication.
13. Karvonen, M. J.: Acta Paediatr. 1949:*37:*68.
14. Karvonen, M. J., and O. Somersalo: Ann. Med. Exp. Biol. Fenn. 1949:*27:*30.
15. Nelson, N.: J. biol. Chem. 1944:*153:*375.
16. Partridge, S. M.: Biochem. J. 1948:*42:*238.
17. Passmore, R., and H. Schlossman: J. Physiol. 1938:*92:*459.
18. Roe, J. H.: J. Biol. Chem. 1934:*107:*15.
19. Schlossman, H.: Erg. Physiol. 1932:*34:*744.
20. Snyder, F. F., and F. M. Hoskins: Anat. Rec. 1928:*38:*28.
21. Somogyi, M.: J. Biol. Chem. 1945:*160:*61.
22. Vartiainen, A. V.: Personal communication.
23. Verzár, F.: Biochem. Z. 1935:*276:*17.

AUS DEM ANATOMISCHEN INSTITUT DER UNIVERSITÄT HELSINKI

ÜBER DEN EINFLUSS DER VERBRENNUNG AUF DIE FETALEN KNOCHENMASSE

Von

ANTTI TELKKÄ

(Eingegangen bei der Redaktion am 6. Oktober 1949)

EINLEITUNG

Die üblichste Weise, sich der Leiche einer gesetzwidrig abgetriebenen Frucht oder eines ermordeten Kindes zu entledigen, dürfte die Verbrennung der Leiche sein. Die verbrannten Knochen, die dem Untersucher zur Analysierung gebracht werden, sind kein sehr leichtes Untersuchungsobjekt, und eine wichtige Aufgabe, die ungefähre Bestimmung der Grösse, also des Alters, bereitet oft Schwierigkeiten. Da das Alter des Kindes sowie der Umstand, ob das Kind lebensfähig gewesen ist oder nicht, in Fällen, wo Verdacht auf Kindermord besteht oder ein solcher ausgeführt worden ist, vom juridischen Standpunkt aus wichtige Bedeutung hat, dürfte es vielleicht angezeigt sein, auf diese Angelenheit etwas näher einzugehen. Frührer hat Schrader (9) über diese Frage auf dem gerichtsmedizinischen Kongress zu Breslau eine Mitteilung gemacht. In seiner auf einem Material von 16 Feten basierenden Untersuchung behauptet er, dass sich bei der Schätzung des Alters auf Grund von verbrannten Knochen mit Hilfe der üblichen Methoden ein Fehler von $1-1\frac{1}{2}$, möglicherweise sogar 2 Monaten ergibt. Untersuchungen über diese Frage gibt es also sehr wenig, weshalb es am Platze ist, sich näher mit der Sache zu befassen.

MATERIAL UND TECHNIK

Das mir zur Verfügung stehende Material stammte aus dem Anatomischen Institut und umfasste 20 Feten (12 ♂ und 8 ♀).

Ihre Scheitel-Fersenlänge war durchschnittlich 46.6 cm (V = 40 —55 cm) und die Scheitel-Steisslänge 32.2 cm (V = 27—38 cm). Um ein zuverlässiges unverbranntes Vergleichsmaterial zu bekommen, wurde bei jedem Fetus das eine Gliedmassenpaar vor der Verbrennung abgetrennt, und zwar abwechselnd rechts und links, dessen Knochen dann als Vergleichsmaterial der anderen Seite präpariert und gemessen wurden. Gliedmassen-Asymmetrien kommen beim Fetus nicht vor (8), oder sie sind dann sehr gering (6), nach Ingelmark (2) bei den verschiedenen Knochen in 85 % unter 0.1 mm, was praktisch genommen bedeutet, dass sie im Messungsfehler enthalten sind, so dass diese Verfahren zur Erzielung eines exakten Vergleichsmaterials berechtigt ist. Untersuchungsobjekte waren die langen Gliedmassenknochen, Humerus, Radius, Ulna, Femur, Tibia und Fibula, von welchen die Längenbestimmung gewöhnlich durchgeführt wird.

Die Verbrennung wurde in einem Zentralheizungsofen bei Holzfeuerung vorgenommen, was den »natürlichen» Verhältnissen entsprechen dürfte. Schrader (9) erwähnt, dass der Umstand, ob Holz-, Steinkohlen- oder Mischfeuerung verwendet wird, keinen Einfluss auf das Resultat habe. Die Verbrennung dauerte ungefähr drei Stunden, wonach nur noch die Diaphysen der Knochen vorhanden waren. Weitere Feuerung schien nur insofern auf sie einzuwirken, als sie noch leichter als früher zerbrachen.

Von den Knochen wurden folgende Masse genommen: Die grösste Länge (nur von den unverbrannten, weil von den verbrannten Knochen, wie schon erwähnt, die Epiphysen nicht mehr vorhanden waren), die Länge der Diaphyse, der Umfang der Diaphysenmitte sowie der transversale Durchmesser der Diaphysenmitte (alle Masse nach Martin) (7).

RESULTATE

Bei der Betrachtung der verbrannten Knochen liess sich feststellen, dass der Humerus im allgemeinen am besten erhalten blieb, desgleichen das Femur, von welchem jedoch oft Fragmente abbröckelten, die Tibia erhielt sich auch ziemlich schön, die Ulna krümmte sich oft, der Radius und die Fibula waren auch recht sauber verbrannt, wegen ihrer Zartheit zerbrachen sie aber oft. In der folgenden Tabelle sind die durchschnittlichen Veränderungen

der Länge aufgeführt. Die Messungen sind mit Genauigkeit von 1 mm ausgeführt und die Resultate in Millimeter angegeben.

TABELLE I.

DIE GANZE LÄNGE UND DIE LÄNGE DER DIAPHYSE VOR DER VERBRENNUNG, DIE DIAPHYSENLÄNGE NACH DER VERBRENNUNG, DIE DURCH DIE VERBRENNUNG BEDINGTE LÄNGENVERMINDERUNG IN DER DER DIAPHYSENLÄNGE IN MM UND %

	Unverbrannt		Verbrannt	Unterschied	
	Ganze Länge	Diaphys.länge	Diaphys.länge	mm	%
Humerus	74.6	60.4	56.8	3.6	6.0
Radius	55.1	47.1	45.2	1.9	4.0
Ulna	63.4	56.8	53.8	3.0	5.2
Femur	89.7	70.8	67.4	3.4	4.7
Tibia	72.6	59.8	57.7	2.1	3.6
Fibula	68.9	57.4	53.2	4.2	7.3

Die folgenden Tabellen enthalten die entsprechenden Angaben über die Umfang der Diaphysenmitte (Tabelle 2) und den transversalen Durchmesser der Mitte (Tabelle 3). Die Resultate sind desgleichen in mm ausgedrückt, und die Messgenauigkeit ist 1 mm.

Durchschnittlich nimmt die Diaphysenlänge des Gliedmassenknochens bei der Verbrennung um 5.1% ab, der Umfang der Diaphysenmitte um 7.3% und ihr transversaler Durchmesser um 10.9%. Zu bemerken ist jedoch, dass beim Messen des Diaphysenumfangs mit dem Millimetermass der Messfehler im Verhältnis zur Kleinheit des Masses beträchtlich ist, und das Gleiche gilt auch bezüglich des Durchmessers, so dass man diesen Resultaten

TABELLE 2.

UMFANG DER DIAPHYSENMITTE

	Unverbrannt	Verbrannt	Untersch. mm	Unterschied %
Humerus	18.6	16.9	1.7	9.2
Radius	12.0	10.9	1.7	9.2
Ulna	13.2	12.1	1.1	8.0
Femur	21.8	20.5	1.3	5.8
Tibia	19.0	18.3	0.7	3.7
Fibula	11.4	10.5	0.9	7.9

TABELLE III.

TRANSVERSALER DURCHMESSER DER DIAPHYSENMITTE

	Unverbrannt	Verbrannt	Untersch.mm.	Unterschied %
Humerus	5.5	4.8	0.7	11.4
Radius	3.8	3.2	0.6	15.2
Ulna	4.4	3.9	0.5	11.5
Femur	6.3	5.8	0.5	8.0
Tibia	5.7	5.3	0.4	7.4
Fibula	3.2	2.9	0.3	11.8

meines Erachtens keinen wesentlichen Wert geben kann. Wahrscheinlich ist es so, dass der Knochen in jeder Beziehung von seinen Massen ungefähr gleichviel einbüsst, und der Verlust des Durchmessers und des Umfangs, welcher grösser ist als die Längenverminderung, ist zum grossen Teil dem Messfehler zuzuschreiben.

DISKUSSION

Nach Schrader bestehen im histologischen Bild des verbrannten und des unverbrannten Knochens deutliche Unterschiede, im verbrannten, calcinierten Knochen ist die Struktur undeutlicher, und die Weite sowie die Anzahl der Havers'schen Kanäle vermindert. Nach Klement und Trömel (5) erklärt sich die Undeutlichkeit der Struktur durch die Veränderung der anorganischen Hauptsubstanz des Knochens, des Hydroxylapatits ($Ca_{10}(PO_4)_6(OH)_2$). Die Hauptursache für die Verkleinerung ist natürlich der Wasserverlust. Nach Schranz (10) beträgt das Gewicht des verbrannten Knochens (bei Erwachsenen) nur 24—31% von demjenigen des frischen Knochens. Die Verkleinerung der Masse beruht also auf dem Wasserverlust und der Veränderung der anorganischen Bestandteile.

Die Hauptfrage, deren Lösung der Zweck dieser Untersuchung war, betraf die Fehler, welche bei der Schätzung der Länge auf Grund von verbrannten Knochen gemacht werden. Kivilaakso und Kehä (4) haben Tabellen ausgearbeitet, mit deren Hilfe die Länge des Fetus auf Grund der Länge der langen Gliedmassenknochen geschätzt werden kann. Wenn man aus meinem Material auf Grund der unverbrannten Diaphysenlänge die Scheitel-Steiss-

länge berechnet, und desgleichen auf Grund der verbrannten, erhält man für die Differenz in der Scheitel-Steisslänge ca 15 mm. Bei den verschiedenen Knochen variiert diese Differenz zwischen 9.7 und 20.5 mm, was im Durchschnitt, wie gesagt, 15 mm ausmacht. Im Alter bedeutet dies, weiterhin nach Kivilaakso und Kehä, bei Feten von dieser Grösse ca 1 ½—2 Wochen. Nach den Tabellen von Kajava (3) entspricht die erwähnte Differenz in der Scheitel-Steisslänge, 15 mm, in der Scheitel-Fersenlänge ca 25—30 mm, was in Alter ausgedrückt desgleichen ungefähr zwei Wochen bedeutet. Da die Genauigkeit derartiger, auf den Knochen beruhender Längenbestimmungen im allgemeinen ausgedrückt im Alter ca. zwei Wochen oder etwas weniger ist, kann die durch die Verbrennung bedingte Veränderung in der Diaphysenlänge nicht als sehr gross angesehen werden. Natürlich muss sie bei der Bestimmung des Alters aus dem verbrannten Knochen berücksichtigt werden, es müssen also zur Länge der verbrannten Diaphyse ca 5% hinzugefügt werden, ehe die Länge anhand von Kivilaakso's und Kehä's oder anderen entsprechenden Tabellen endgültig geschätzt wird.

Die von Schrader mitgeteilten Resultate weichen von den meinigen ziemlich viel ab, sein im Alter ausgedrückter Unterschied von 1—1 ½ Monaten würde einem Längenverlust von ca. 10—15%, ja sogar 20% entsprechen. Absolute Zahlenwerte führt er in seiner Mitteilung nicht an, sondern verweist auf eine Publikation von Bunsen (1), welche ich leider nirgends auftreiben konnte nicht einmal als Referat. Vielleicht sind die Unterschiede durch die Messungstechnik und die Genauigkeit bedingt, aber auf Grund meines eigenen Materials glaube ich behaupten zu können, dass die durch die Verbrennung verursachte Verkürzung auf keinen Fall mehr als 10% beträgt, eher noch weniger. Eine ähnliche Zahl gibt Werkgartner (11) in seiner kasuistischen Veröffentlichung an.

ZUSAMMENFASSUNG

Der Verfasser hat den Einfluss der Verbrennung auf die fetalen Knochenmasse untersucht. Das Material bildeten 20 Feten, deren durchschnittliche Länge 46.6 cm betrug. Untersuchungsobjekt waren die langen Gliedmassenknochen, welche von der einen Körperseite als Vergleichsmaterial präpariert und von der ande-

ren verbrannt wurden. Die Verbrennung wurde bei Holzfeuerung in einem Zentralheizungsofen vorgenommen. Der Längenverlust in der Diaphyse war durchschnittlich 5.1%, der Umfang der Diaphysenmitte nahm durchschnittlich um 7.3 % ab, und ihr transversaler Durchmesser durchschnittlich 10.9%. Bei den zwei letztgenannten Massen wirken jedoch die Messungsfehler beträchtlich. Bei der Bestimmung der Länge auf Grund des verbrannten Knochens müssen zum Mass also ca. 5% hinzugefügt werden. Die Resultate weichen von den von Schrader (9) mitgeteilten etwas ab, aber auf Grund seines eigenen Materials glaubt der Verfasser behaupten zu können, dass der Längenverlust nicht über 10% beträgt, eher weniger.

LITERATURVERZEICHNIS

1. Bunsen, A.: Zit. n. Schrader.
2. Ingelmark, B.: Upsal. Läkareför. Förh. N. F. 1943:*48:*227.
3. Kajava, Y.: Duodecim. 1927:*9:*683.
4. Kivilaakso, R. and Kehä, H.: Duodecim. 1942:*2:*66.
5. Klement, R. and Trömel, G.: Z. physiol. Chem. 1932:*213:*263.
6. König, K. and Kornfeld, W.: Z. Anat. Entw. gesch. 1927:*82:*657.
7. Martin, R.: Lehrbuch der Anthropologie. 2 Aufl. Jena 1928.
8. Moorhead, T. G.: J. Anat. a. Physiol. 1902:*36:*400.
9. Schrader, G.: Dtsch. Z. ges. gerichtl. Med. 1938:*29:*152.
10. Schranz, D.: Dtsch. Z. ges. gerichtl. Med. 1933:*22:*332.
11. Werkgartner, A.: Dtsch. Z. ges. gerichtl. Med. 1938:*29:*158.

FROM THE CHILDREN'S CLINIC, UNIVERSITY OF HELSINKI

SODIUM AND POTASSIUM IN ADULT AND FOETAL SHEEP ERYTHROCYTES

By

N. HALLMAN and M. J. KARVONEN

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The red cells of the mammals can be divided into two groups. In one group (man, monkey, rat, rabbit, guinea pig, pig) the erythrocytes have a high concentration of potassium and a low level of sodium, which corresponds with the other cells of the animal body in general. In the other group (horse, cattle, dog, cat), sodium is the main cation of the erythrocytes (1, 9). The values have been determined generally on adult and sometimes on growing animals. It has been inferred that in young calves, the intracellular potassium values are most probably higher than in adult cattle (4, 12). To our knowledge, no similar studies have been performed on any foetal erythrocytes. It was deemed desirable to get some information also on them.

Foetal sheep blood was obtained in connection with other studies on sheep foetuses. A number of sodium and potassium determinations were performed on the plasma and the erythrocytes. For adult sheep, there are in the literature the results of two analyses performed by Abderhalden (1) already in 1895. According to his values the erythrocytes contain 15.7—15.9 milliequivalent of potassium per kg, and 79.4—88.5 m.equiv. of sodium per kg. The corresponding values for serum were: K 5.45—5.52 m.equiv., and Na 138—140 m.equiv. per kg. Kerr (9) found a great indivi-

dual variation among sheep belonging to a mixed breed: the intracellular potassium varied between 18.4 and 64.2 m.equiv. per kg, and the sodium values were correspondingly from 83.5 to 15.6 m.equiv. per kg. The values for adult sheep were determined also in the course of the present work; the results of these determinations differed from those of both Abderhalden and Kerr.

MATERIAL AND METHODS

Sheep of the Finnish standard breed were used. The foetal ages were not exactly known, but they were assessed approximately with the aid of the length-age and weight-age curve (3) for Welsh mountain sheep, a breed much resembling that used in the present work.

The maternal blood samples were taken either before the anaesthesia from the jugular vein or during a nembutal anaesthesia from the uterine vessels. No difference was observed between the samples taken before and during the anaesthesia. The samples from non-pregnant adult animals were taken without anaesthesia. The foetal samples were drawn from the umbilical vessels, during anaesthesia.

The samples were taken with a syringe and kept under paraffin oil. The coagulation was prevented with the aid of crystalline heparin. The samples were immediately centrifuged for 30 min. at 3,000 r. per min., and the plasma was separated with a pipette. The pipetting was repeated 10 min. after the centrifugation. The erythrocytes were pipetted into volumetric bottles by using specially calibrated pipettes with wide tips; the cells were haemolyzed, when the pipettes were washed with distilled water.

The sodium and potassium determinations were carried out with a flame photometer of own design (5). The apparatus is of the 'internal standard' type. The instruments error was found to be ± 1.8 per cent for potassium and ± 1.4 per cent for sodium.

RESULTS

The results of the analyses of the foetal and maternal samples are compiled in the form of a table (Table I). Standard deviations have been calculated for the averages. However, the results are

TABLE I

SODIUM AND POTASSIUM (MILLIEQUIVALENTS PER LITRE) IN MATERNAL AND FOETAL SHEEP PLASMA AND ERYTHROCYTES

No. of Sheep	Crown-rump Lenght of Foetus	Foetal Age Days (appr.)	Plasma				Erythrocytes			
			Sodium		Potassium		Sodium		Potassium	
			Mother	Foetus	Mother	Foetus	Mother	Foetus	Mother	Foetus
H 23	13 cm	62	145	148	H*	5.2	35	12	87	100
H 26	28 cm	95	143	145	5.4	4.7	29	17	93	103
H 27	28 cm	98	146	146	4.7	4.7	31	17	95	103
H 29	35 cm	115	146	(136?)	4.9	5.4	24	16	95	107
H 30	38 cm	125	146	145	4.5	5.0	27	18	85	105
Averages:			145.2	146.0	4.88	5.00	29.2	15.6	91.0	103.6
Standard deviation			±0.4	±1.4	±0.43	±0.31	±4.2	±2.3	±4.7	±2.6

* Haemolysis.

TABLE II

SODIUM AND POTASSIUM (MILLIEQUIVALENTS PER LITRE) IN NON-PREGNANT SHEEP PLASMA AND ERYTHROCYTES

Sheep	Plasma		Erythrocytes	
	Sodium	Potassium	Sodium	Potassium
Lamb, one month old.....	142	7.5	20	114
Non-pregnant ewe	146	4.8	27	103
Ram	142	6.1	24	96

quite consistent, and hardly need any statistical treatment. The Na and K levels in the serum of the mother and foetus were identical. The intracellular Na values of the foetal blood were less than those of the maternal blood, and the K values of the foetal red cells were correspondingly higher.

The results of a few determinations on samples from non-pregnant sheep are given in Table II. These results agree approximately with the above values for maternal sheep.

DISCUSSION

The present values for the concentrations of potassium — 4.4 to 5.4 m. equiv./l. — and of sodium — 143 to 146 m.equiv.l. — in the *serum of adult sheep* are in an agreement with Abderhalden's

values. (His results were expressed per unit weight.) However, the values for the *erythrocytes* — 85 to 94 m.equiv. sodium per litre — differ from previous values. The proportion of these two electrolytes in the present series is opposite to that found by Abderhalden. No explanation can be offered for this discrepancy. The method used in the present work yields on human erythrocytes values which agree with those given by other workers; sheep red cells are so far the only instance of a divergence from previous results.

In *foetal blood*, the potassium and sodium gradients between the erythrocytes and the serum are higher than in the blood of the mother. The unequal distribution of these electrolytes between plasma and cells is at present understood to depend on an *active process*, and not on a simple impermeability. At least one of these electrolytes is being actively transferred across the cell membrane, and the observed distribution expresses a dynamic equilibrium between active transfer, osmotic leakage along the gradient, and Donnan equilibrium (10).

The active transfer of electrolytes against the gradient has been connected by Harris (6) with the glycolytic *metabolism* of the erythrocytes. The steep gradients in the foetal blood may well depend on the fact that the rate of glycolytic and oxidative metabolism of the foetal sheep erythrocytes is higher than that of the maternal red cells (8). More energy is released, and so far as we know, its only immediate use is osmotic work; finally, of course, all the energy produced by the red cells is dissipated from them as heat.

According to modern views, the electrolyte distribution does not necessarily give any direct information on the *permeability* of the cell membrane. Moreover, there are only very few studies, where the permeability of foetal and adult erythrocytes towards to any substance have been compared. Hitchcock (7) has studied the distribution of sugars in the blood of sheep; in foetus the corpuscle/plasma ratio of total sugar was not far from unity, but the ratio decreased within the first month after birth to values of the order of 0.3 The foetal erythrocytes were thus more permeable to sugar than those of the adult. A corresponding difference was found by Andreen-Svedberg (2) in a study of the sugar concentrations in the plasma and erythrocytes of a 'very young' calf and of an adult cow: in the former the ratio was 0.59, and in the latter

0.26. If there is an analogous difference in the permeability towards sodium and potassium, the high foetal gradients obviously depend on a more intense active transfer by the cell membrane.

The water content of the foetal body is quite high, and it gets reduced with increasing age. At the same time the potassium concentration of the body decreases gradually with advancing age (11). These variations in the electrolytes have been ascribed to changes in the proportions of extracellular and intracellular fluid. However, the present work shows that the possibility of variations in the composition of the intracellular fluid must obviously also be taken into account in calculating the relative amounts of extracellular and intracellular fluid. In order to do it exactly, the composition of the intracellular fluid proper ought to be known. Owing to the exceptional nature of the erythrocytes, a parallel behaviour does not necessarily obtain.

SUMMARY

1. The potassium and sodium concentrations of plasma and erythrocytes were determined in the blood of adult and foetal sheep by using flame photometric technique.

2. The average results as milliequivalents per litre were:

Plasma: Sodium: Pregnant ewes: 145.2±0.4 Foetuses: 146.0±1.4.
Potassium: » » 4.88±0.43. » 5.00±0.31.
Erythr. Sodium: Pregnant ewes: 29.2±4.2. Foetuses: 15.6±2.3.
Potassium: » » 91.0±4.7. » 103.6±2.6..

The results for non-pregnant ewe and ram were analogous with those for pregnant ewe.

3. The values for adult sheep differ from those reported previously.

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REFERENCES

1. Abderhalden, E.: Z. physiol. Chem. 1898:*25*:65.
2. Andreen-Svedberg, A.: Skand. Arch. Physiol. 1933:*66*:113.

3. BARCROFT, J.: Researches on Pre-natal Life. 1946. Blackwell Scientific Publications. Oxford.
4. GREEN, H. H. and E. H. MACASKILL: J. Agr. Sci. 1928: *18:* 384.
5. HALLMAN, N. and V. LEPPÄNEN: In press.
6. HARRIS, J. E.: J. Biol. Chem. 1941:*141:*579
7. HITCHCOCK, M. W. S.: J. Physiol. 1949:*108:*117.
8. KARVONEN, M. J.: Acta Physiol. Scand. 1949:*17:*267.
9. KERR, S. E.: J. Biol. Chem. 1937:*117:*227.
10. KROGH, A.: Proc. Roy. Soc. London, B. 1946:*133:*140.
11. SHOHL, A. T.: Mineral Metabolism. 1939: International Textbook Press. Scranton, PA.
12. WISE, G. H., M. J. CALDWELL, D. B. PARRISH, R. J. FLIPSE and J. S. HUGHES: J. Dairy Sci. 1947: *30:* 983.

FROM THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF HELSINKI

EFFECT OF STORAGE ON THE ANTIDIURETIC AND PRESSOR ACTIVITIES OF POSTERIOR PITUITARY EXTRACT

IS THE ANTIDIURETIC PRINCIPLE IDENTICAL WITH VASOPRESSIN OR IS IT A SEPARATE HORMONE?

By

OSMO VARTIAINEN and EINO V. VENHO

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Oliver and Schäfer noticed in 1895 that intravenous injection of pituitary extract caused a rise in blood pressure, and a few years later Howell (1898) found that the source of this action was the posterior lobe of the pituitary gland, which subsequently became the object of intense study. It was soon discovered that posterior pituitary extract had other distinct actions, too. It was found to check the secretion of the urine and cause contractions in the uterus (Magnus and Schäfer 1901, Dale 1906).

It remains a moot question whether one or several chemical substances are responsible for these actions. There are investigators who think that the oxytocic, vasopressor, and antidiuretic actions are all due to the presence of a single substance (Guggenheim 1914, 1917, Smith and McClosky 1924, Abel, Rouiller, and Geiling 1934).

To these three distinct actions of posterior pituitary extract some writers add diuretic activity, one or several pressor and depressor components, and a respiratory component. Watanabe and Crawford (1916) thought that posterior pituitary extract had one or two depressor components and one or several pressor components. Dudley (1923) also presumed that there were two pressor principles. The depressor activity has been ascribed to histamine.

Present-day authorities believe that posterior pituitary extract contains at least two principles. That responsible for uterine contractions is called oxytocin or α-hypophamin and that responsible for rise in blood pressure is called vasopressin or β-hypophamin. It has been possible to separate, from posterior pituitary extract, a fraction of which one mg contains ca. 250 units of oxytocin and only five units of vasopressin. It has also been possible to separate a fraction of which one mg contains ca. 200 units of vasopressin and ca. 10 units of oxytocin (Stehle and Fraser 1935). But attempts to separate the antidiuretic principle from the pressor principle have not been successful (Selye 1947). It has been suggested that a separate antidiuretic principle does not exist at all and that it is to be identified with vasopressin (Kamm, Aldrich, Grote, Rowe, and Bugbee 1928, Stehle 1934, and Fraser 1941).

So far, it has not been possible to isolate the pure hormones, and their chemical composition remains obscure, but it has been found that the chemical and physical properties of oxytocin and vasopressin are alike (Stehle 1938, Rosenfeld 1940). Smith and McClosky (1925) demonstrated parallel destruction of the oxytocic and pressor activity in heat and thus claimed to have the evidence for the view advanced by Abel in favour of chemical unity.

On the other hand, there are writers who think that the antidiuretic effect is due neither to the pressor nor to the oxytocic principle (Bijlsma, Burn, and Gaddum 1928, Heller 1939).

It has been experimentally proved that under given conditions posterior pituitary extract does not always lose its effect in the same way. Adams (1917) noticed that when pituitary extract was heated in *p*H 5, it lost its oxytocic effect to a considerable extent. Stasiak (1926) found that 0.5 per cent hydrochloric acid and more than 6 per cent acetic acid in the solution destroyed the oxytocic activity of the extract. Kamm and co-workers noticed that the reaction of pituitary extract prepared without acetic acid approximated *p*H 6 and that it lost its pressor activity in heat to a still greater extent than its oxytocic activity. Heller (1939) showed that the oxytocic factor was more stable than vasopressin and the antidiuretic factor within the limits of *p*H 2.0 and 4.5 and less stable outside this *p*H range, and that the antidiuretic factor was more stable than the vasopressor factor at all *p*H values between 0.57 and 10.0. Fraser (1941) thought that in Heller's experiments there was no question

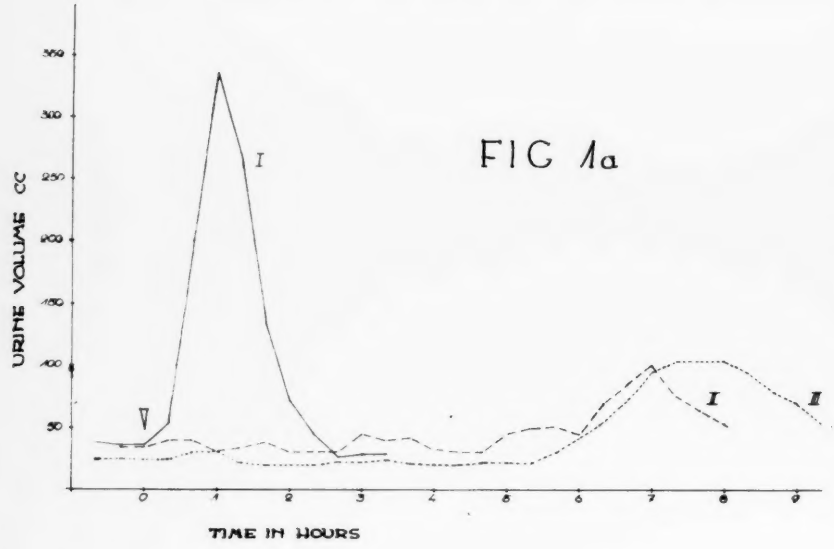
of an antidiuretic principle at all. He thought that the prolongation of the antidiuretic action was due to the subcutaneous injection of some substances produced at hydrolysis. This, however, could not be demonstrated when intravenous injection was used.

The starting-point for the investigation here reported was the assumption that the different constituents of posterior pituitary extract do not lose their activity in the same way, so that in long-stored preparations one type of activity is probably better preserved than another. Assuming further that in a given preparation the vasopressor activity and the antidiuretic activity can be clearly differentiated from one another in this respect, this ought to show that they are two separate substances. On the other hand, if they are one single substance, the decrease in the pressor and antidiuretic effects ought to be parallel.

We studied the vasopressin content and antidiuretic potency in the posterior pituitary preparations of five pharmaceutical houses (*Pituglandol*, F. Hoffman — La Roche & Co.; *Physormon*, Chemische Fabrik Promonta G.m.b.H., Germany; *Pituitrin*, Parke, Davis & Co., England; *Hypadrin*, Astra, Sweden; *Hypoitrin*, Orion, Finland). We tested altogether nine different preparations, the oldest being stored for about fifteen years and the most recent ones for less than two.

The animals used for the determination of the antidiuretic potency have been the dog, rabbit, rat, and mouse (Glaubach and Molitor 1932). Gibbs (1930) was the first to use the mouse for these experiments, but Burn (1931) regarded this animal as of too small size and the determination of its volume of urine therefore as so inaccurate that he carried out his experiments on rats. Bijlsma, Burn, and Gaddum (1928) used human subjects for the determination of the antidiuretic effect. Though expedient for special scientific purposes, this is scarcely practicable as a general method.

We tested the antidiuretic effect of these preparations on normal subjects. The subjects had had some porridge and a glass of milk or some tea on the same morning. The volume of urine excreted was measured at intervals of twenty or thirty minutes until it remained the invariable within a given time unit. After this, the subjects were administered one litre of tepid water, and the volume of urine was measured again at twenty or thirty minute intervals. In case of normal water tolerance the volume of urine



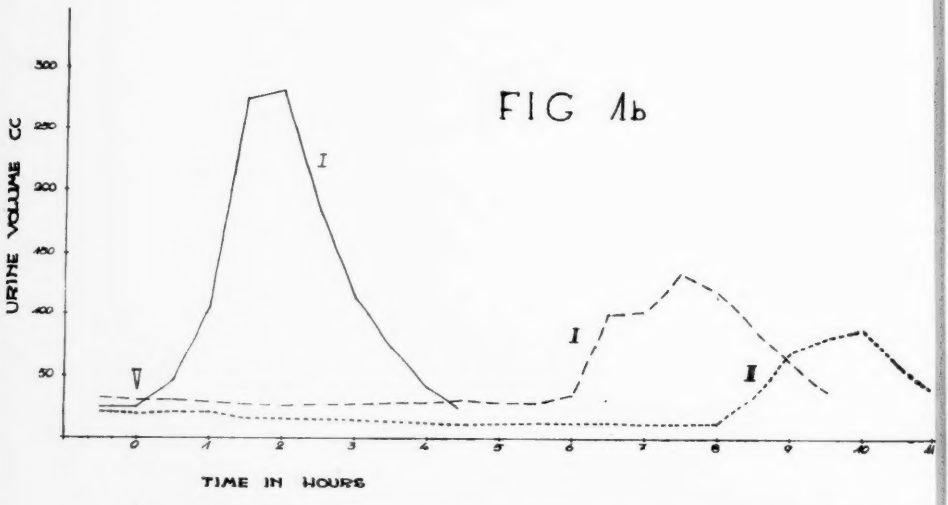


Fig. 1, a and b. — Water tolerance curves of two subjects, A and B.

I. Simple water tolerance test: the subject was given one litre of tepid water (↓).

II. Water tolerance test in which the subject, who had taken one litre of tepid water (↓), received simultaneously an intramuscular injection of 5 units of Physormon, stored for approximately 15 years.

III. Water tolerance test in which the subject took one litre of tepid water (↓) and received simultaneously an intramuscular injection of 5 units of Hypadrin stored for two years and a half.

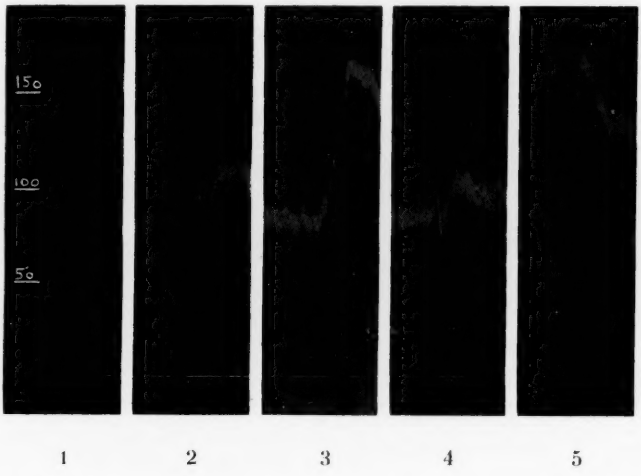


Fig. 1 c. — Determination of the vasopressin content on a decerebrated cat weighing 5.5 kg. The cat received injections of posterior pituitary extract at half-hour intervals. (1), (3), and (5): one unit of Hypadrin; (2) and (4): two units of Physormon.

It is evident from the series that there is a considerable difference between the pressor activity and antidiuretic potency. The antidiuretic activity of Physormon stored for approximately fifteen years shows perhaps some decrease in comparison with Hypadrin stored only two years and a half, but its vasopressin content has decreased much more. Two units of Physormon are not sufficient to raise the blood pressure more than one-third of the rise caused by one unit of Hypadrin. It is therefore very probable that the vasopressin content of Physormon is less than one-fourth of that of Hypadrin.

was reduced to normal limits within three or three and a half hours, the peak of excretion being reached ca. one or one and a half hours after the administration of the water. The test was then repeated on the same subjects, but the subjects received intramuscular injections of posterior pituitary extract simultaneously with the administration of water. This time, the excretion of the urine was delayed depending on the subject, dosage, and length of storage. A total of twenty-six tests were carried out (Figs. 1 a and b, 2 a and b, 3 a and b). There was an interval of one or two days between the tests carried out on the same individual.

It was found that the antidiuretic effect of preparations stored for approximately the same length of time was not the same. It also happened that earlier preparations of one house had a more powerful antidiuretic effect than their later ones. As a rule, it is

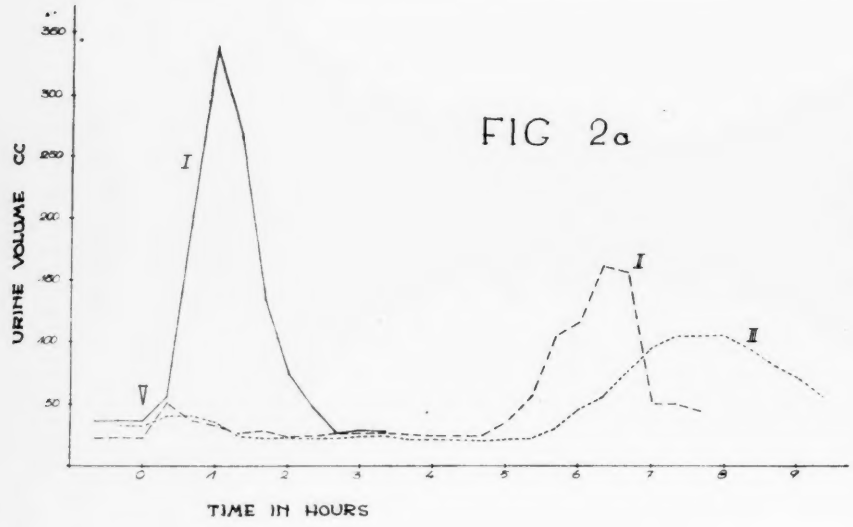


Fig. 2, a and b. Water tolerance curves of two subjects. A and B.

I. Simple water tolerance test in which the subject took one litre of tepid water (↓).

II. Water tolerance test in which the subject took one litre of tepid water (↓) and received simultaneously an intramuscular injection of five units of Pituitrin stored for approximately eleven years.

III. Water tolerance test in which the subject took one litre of tepid water (↓) and received simultaneously an intramuscular injection of five units of Hypadrin stored for approximately two years and a half.

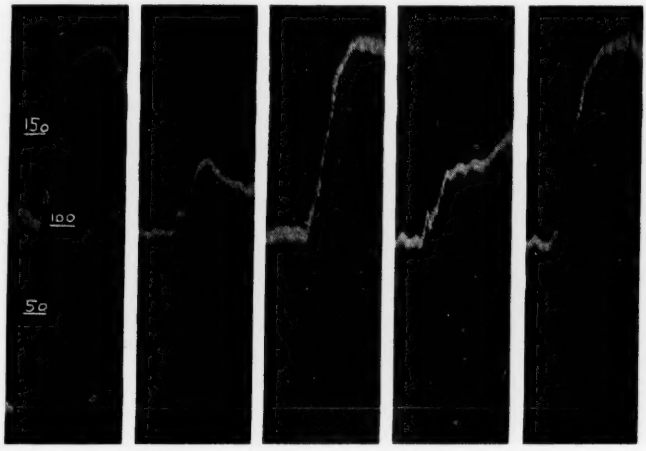


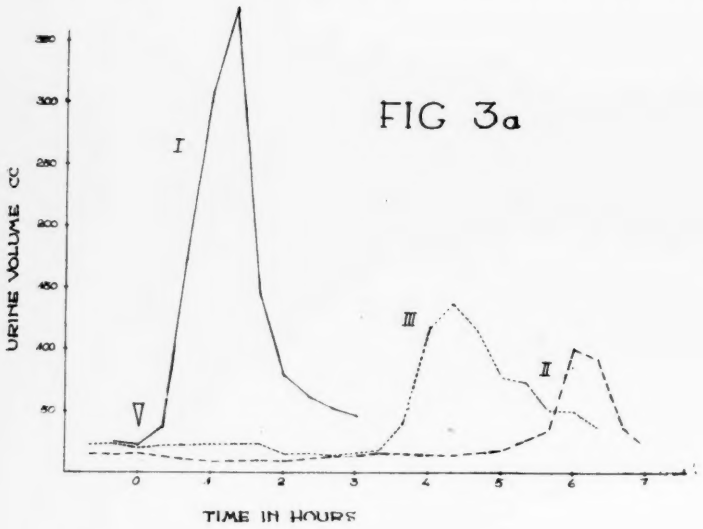
Fig. 2 c. — Determination of the vasopressin content on a decerebrated cat weighing ca. five kg. The cat received injections of posterior pituitary extract at intervals of forty minutes. (1), (3), and (5): one unit of Hypadrin; (2) and (4): one unit of Pituitrin.

The series shows that the antidiuretic potency of Pituitrin is approximately as great as that of Hypadrin, while its vasopressin content might, at the most, be only half of that of Hypadrin. Thus there is a clear difference between the pressor activity and the antidiuretic potency.

considered sufficient to assay the posterior pituitary preparations only for their oxytocic activity, because it is assumed that this is parallel with the pressor activity and the antidiuretic potency. It is doubtful, however, whether this is true of all preparations (Burn 1931, Wokes 1932). Wokes, on the basis of his experiments, thinks that »the oxytocic activity of a pituitary extract is *not* a safe guide to its antidiuretic potency. Extracts which are to be employed for the antidiuretic effect must be assayed for this activity.»

The specific gravity of the urine, which, in the course of a simple water tolerance test, fell very nearly to 1,000, to return to normal when the urine volume did so, remained normal or showed slight rise at the beginning of the water tolerance test combined with injection of pituitary extract, and returned to normal when the excretion began.

The subjects received, as a rule, five Voegtlin units of posterior pituitary extract. Some preparations inhibited the secretion of



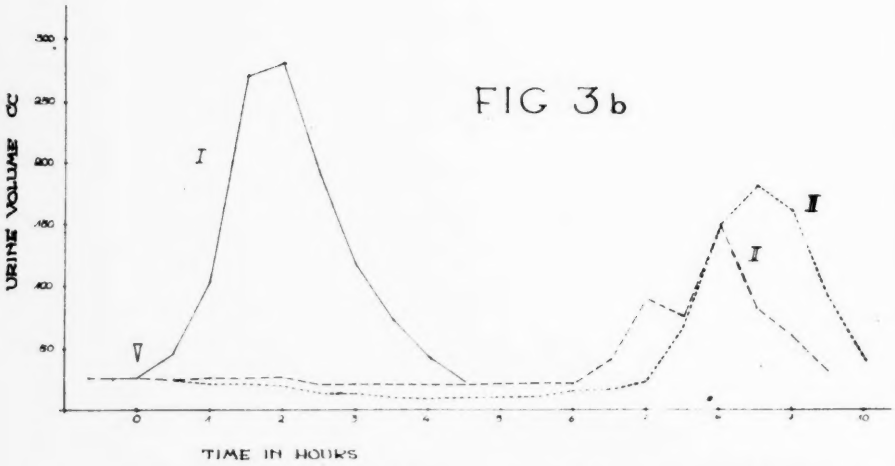


Fig. 3, a and b. Water tolerance curves of two subjects, A and B.

I. Simple water tolerance test in which the subject took one litre of tepid water (↓).

II. Water tolerance test in which the subject took one litre of tepid water (↓) and received simultaneously an intramuscular injection of five units of Pituglandol stored for approximately nine years.

III. Water tolerance test in which the subject took one litre of tepid water (↓) and received simultaneously an intramuscular injection of five units of Hypadrin stored for approximately three years.

Fig. 3 c. — Determination of the vasopressin content on a decerebrated cat. The cat received injections of posterior pituitary extract at intervals of forty-five minutes. (1), (3), and (5): one unit of Hypadrin; (2), (4), (6), and (8): one unit of Pituglandol; (7): half a unit of Hypadrin.

The series shows that the antidiuretic potency of these preparations is roughly the same, while the vasopressin content of Pituglandol is only half of that of Hypadrin.

urine for as much as eight to ten hours. We carried out a couple of tests in which the subjects were first administered five units of Hypoitrin (Orion). This inhibited the excretion of the urine for seven to nine hours. Two days later the subjects received three units of the same preparation, and this inhibited the secretion of urine for three to four and a half hours. This shows that there is a clear correlation between the delay in the secretion of urine and the dosage. Comparatively soon after the beginning of the test the subjects noticed an increase in the discharge of saliva. About four hours later they became aware of a heavy feeling in the head and of incipient headache, which continued until the secretion of urine began. The blood pressure of the subjects was measured, but it showed no definite changes. In some cases abdominal pain was present. During the test, with the volume of the urine continuing to be small, micturition was easier than before the administration of the extract.

Determination of vasopressin has been carried out on dogs, which have been anesthetised by intraperitoneal administration of chloretone (trichlor-tertiarybutyl-alcohol) in oil solution (Hamilton 1912, Hamilton and Rowe 1916, Rowe 1917). Hodgen, Schlapp, and

McDonald (1924), used decerebrated cats and achieved an accuracy of ca. 7—10 per cent. At intervals of an hour or half an hour or so, depending on the response, the cats received alternate injections of the solution to be tested and control solution. Our tests were likewise carried out on decerebrated cats.

The same preparations which had been tested for antidiuretic potency were tested for vasopressin content. As seen from Figs. 1 c, 2 c, and 3 c, the long-stored preparations had lost a considerable proportion of their vasopressor activity. The experiments showed further that in the oldest preparations there was a remarkable difference between the vasopressor activity and antidiuretic potency. Fig. 1, for example, shows that although the vasopressin content of Physormon is less than one-fourth of that of Hypadrin, there is no marked difference in their antidiuretic potencies. This was revealed by the other tests, too; yet, the shorter the period of storage, the less obvious was the difference.

It has to be admitted that the number of preparations tested was not very large; there were nine preparations manufactured by five pharmaceutical houses. The results suggested that the antidiuretic potency of the extract possibly is not due to vasopressin alone and that a separate antidiuretic principle may exist.

CONCLUSIONS

We carried out experiments with fresh and long-stored posterior pituitary extracts of various pharmaceutical houses and determined their antidiuretic potency and vasopressin content. It was found that even in fresh preparations with standardised oxytocic activity the antidiuretic potency showed considerable variations. When the preparations are kept in store, the pressor activity disappears more rapidly than does the antidiuretic potency. This suggests that the antidiuretic activity is not due to vasopressin, but to a separate antidiuretic principle.

BIBLIOGRAPHY

ABEL, JOHN J., ROUILLER, CHAS A., and GEILING, E. M. K.: J. Pharmacol. & Exper. Therap. 1924:*22:*289.

ADAMS, H. S.: J. Biol. Chem. 1917:*30:*235.

BIJLSNA, U. G., BURN, J. H., and GADDUM, J. H.: Quart. J. Pharm. & Pharmacol. 1928:*1:*493.

BURN, J. H.: Quart. J. Pharm. & Pharmacol. 1931:*4:*517.
DALE, H. H.: J. Physiol. 1906:*34:*163.
DUDLEY: J. Pharmacol. & Exper. Therap. 1923:*21:*103.
FRASER, A. M.: J. Physiol. 1941:*100:*233.
GIBBS, O. S.: J. Pharmacol. & Exper. Therap. 1930:*40:*129.
GLAUBACH, SUSI, and MOLITOR, HANS: Arch. f. exper. Path. u. Pharmakol. 1932:*166:*243.
GUGGENHEIM, M.: Biochem. Ztschr. 1914:*65:*189, 1917:*81:*274.
HAMILTON, H. C.: J. Am. Pharm. A. (Scient. Ed.) 1912:*1:*1117.
HAMILTON and ROWE: J. Lab. & Clin. Med. 1916:*2:*120: quoted by J. Pharmacol. & Exper. Therap. 1917:*9:*107.
HELLER, H.: J. Physiol. 1939:*96:*337.
HODGEN, LANCELOT T., SCHLAPP, WALTER, and MACDONALD, A. D.: Quart. J. Exper. Physiol. 1924:*14:*301.
HOWELL, W. H.: J. Exper. Med. 1898:*3:*245.
KAMM, OLIVER, ALDRICH, T. B., GROTE, I. W., ROWE, L. W., and BUGBEE, E. P.: J. Am. Chem. Soc. 1928:50_1:573.
MAGNUS, R., and SCHÄFER, E. A.: J. Physiol. 1901:*27:*IX.
OLIVER, G., and SCHÄFER, E. A.: J. Physiol. 1895:*18:*277.
ROSENFELD, MORRIS: Bull. John Hopkins Hosp. 1940:*66:*398 quoted by Ber. ü.d. ges. Physiol. u. exper. Pharmakol. 1941:*122:*625.
ROWE, L. W.: J. Pharmacol. & Exper. Therap. 1917:*9:*107.
SELYE, HANS: Textbook of Endocrinology 1947, Acta Endocrinologica Université de Montréal, Canada.
SMITH, MAURICE I., and MCCLOSKY, WM. T.: Hygienic Lab. Bull. 1924: No 138:1.
SMITH, MAURICE I., and MCCLOSKY, WM. T.: J. Pharmacol. & Exper. Therap. 1925:*24:*391.
STASIAK, A.: J. Pharmacol. & Exper. Therap. 1926:*28:*1.
STEHLE, R. L.: Arch. f. exper. Path. u. Pharmacol. 1934:*175:*471. Ergebn. d. Vitamin- u. Hormonforsch. 1938:*1:*114.
STEHLE, R. L., and FRASER, A. M.: J. Pharmacol. & Exper. Therap. 1935: *55:*136.
WATANABE, WALTER K., and CRAWFORD, ALBERT C.: J. Pharmacol. & Exper. Therap. 1916:*8:*75.
WOKES, FRANK: Quart. J. Pharm. & Pharmacol. 1932:*5:*390.